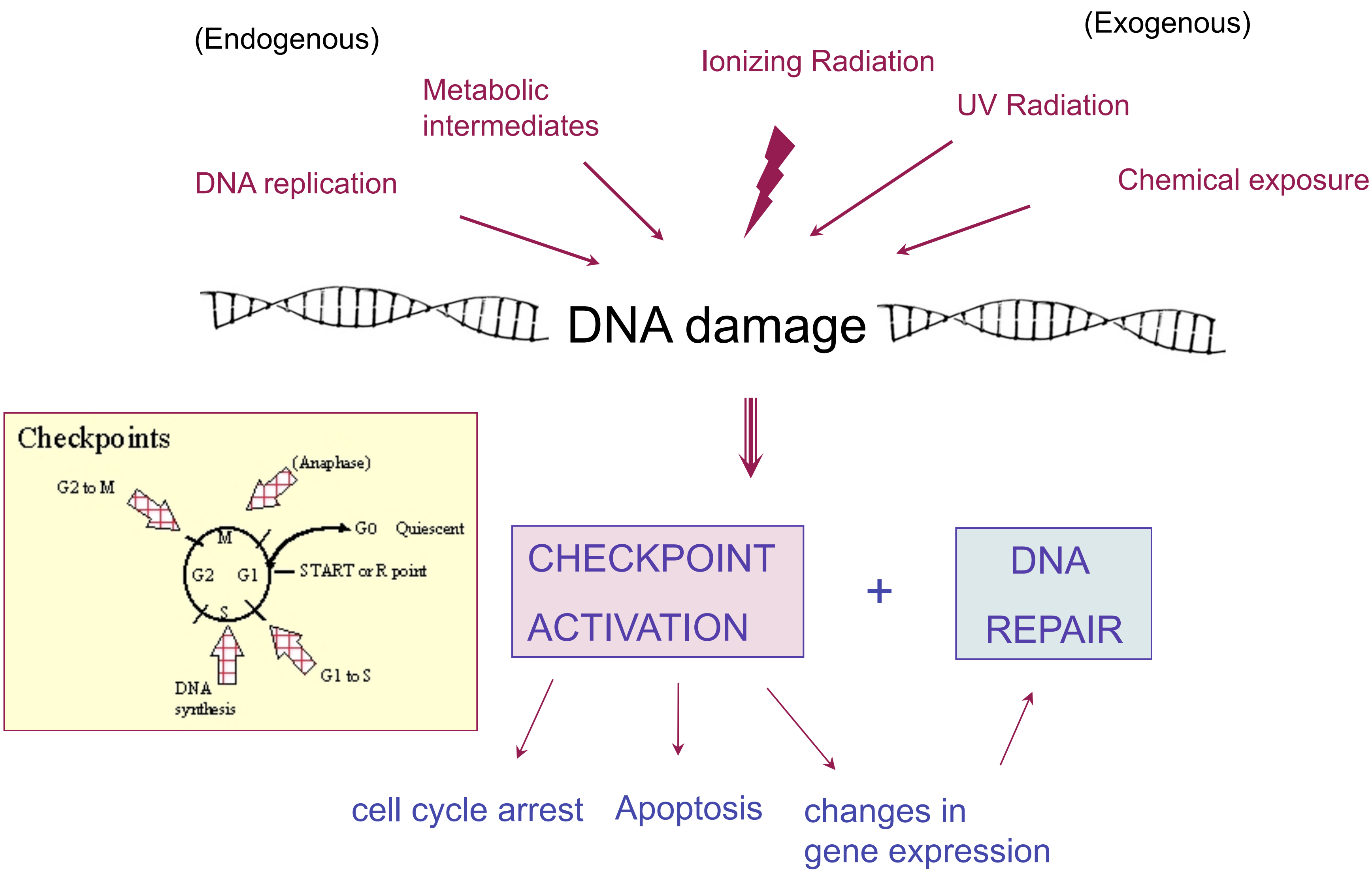
The Mre11/Rad50/Nbs1(Xrs2) complex and DNA double-strand break processing

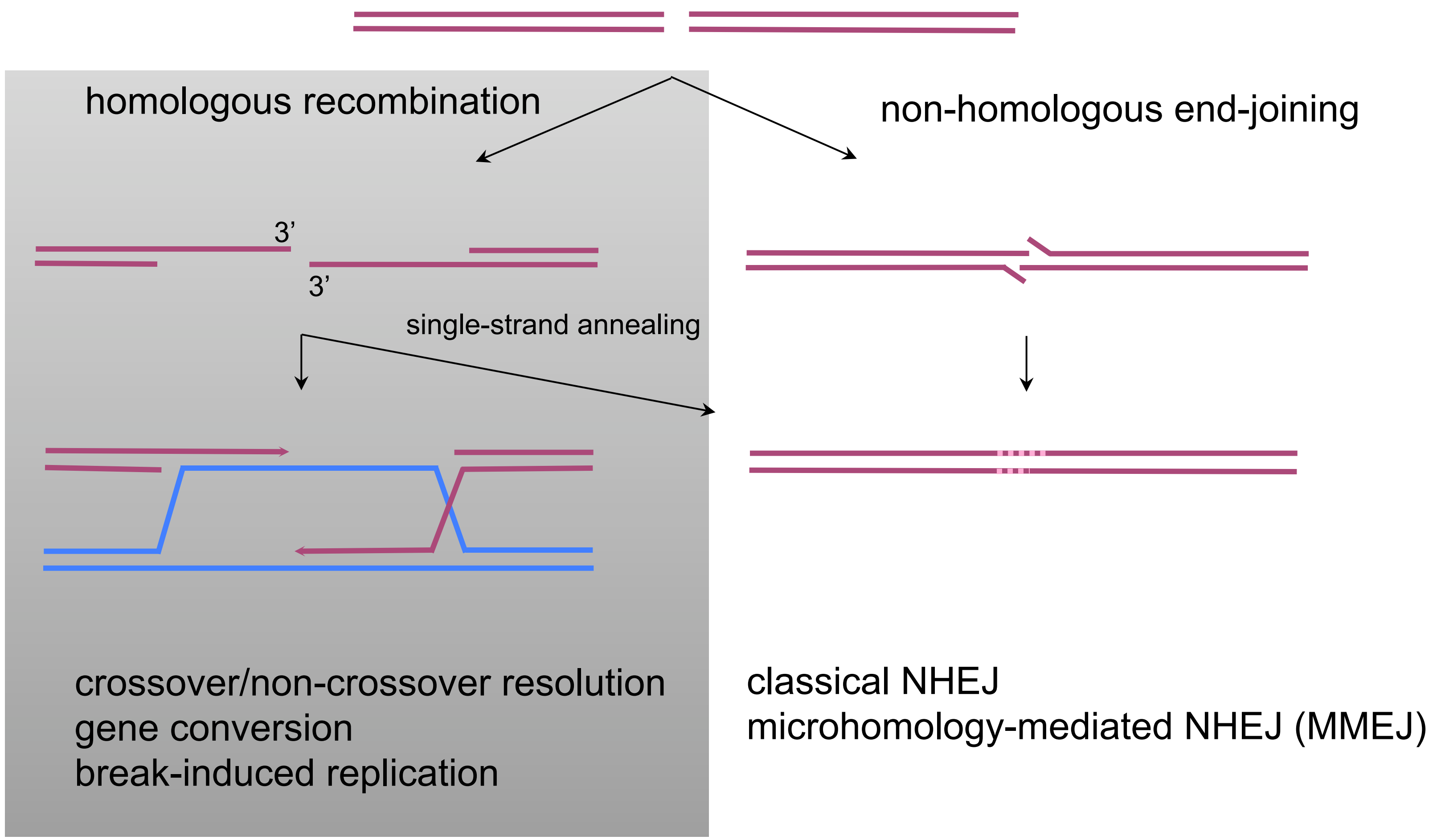
1. archaea
2. budding yeast

Tanya Paull
MD Anderson Science Park/ NIH videoconference
12/08

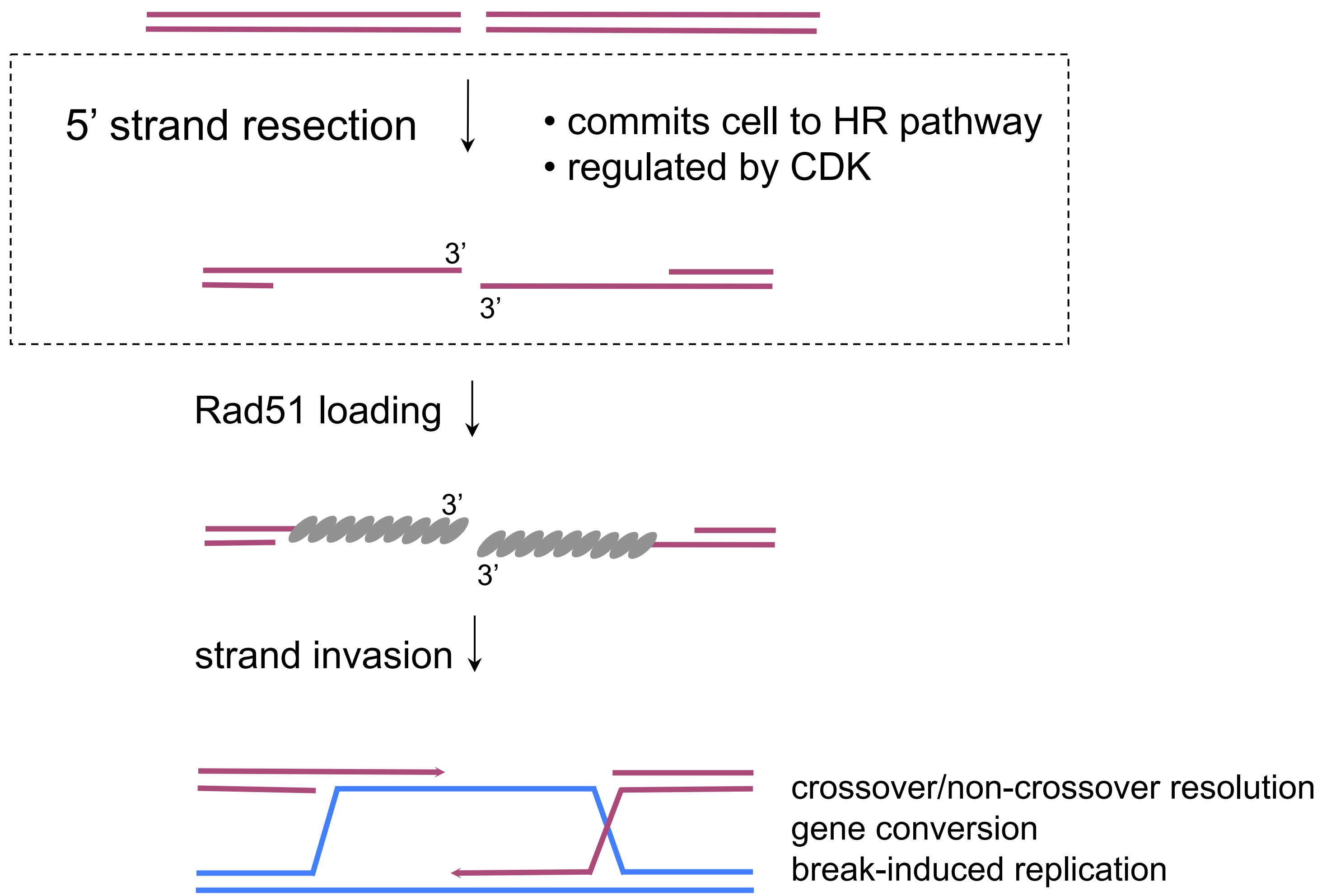
The DNA damage response in eukaryotic cells:



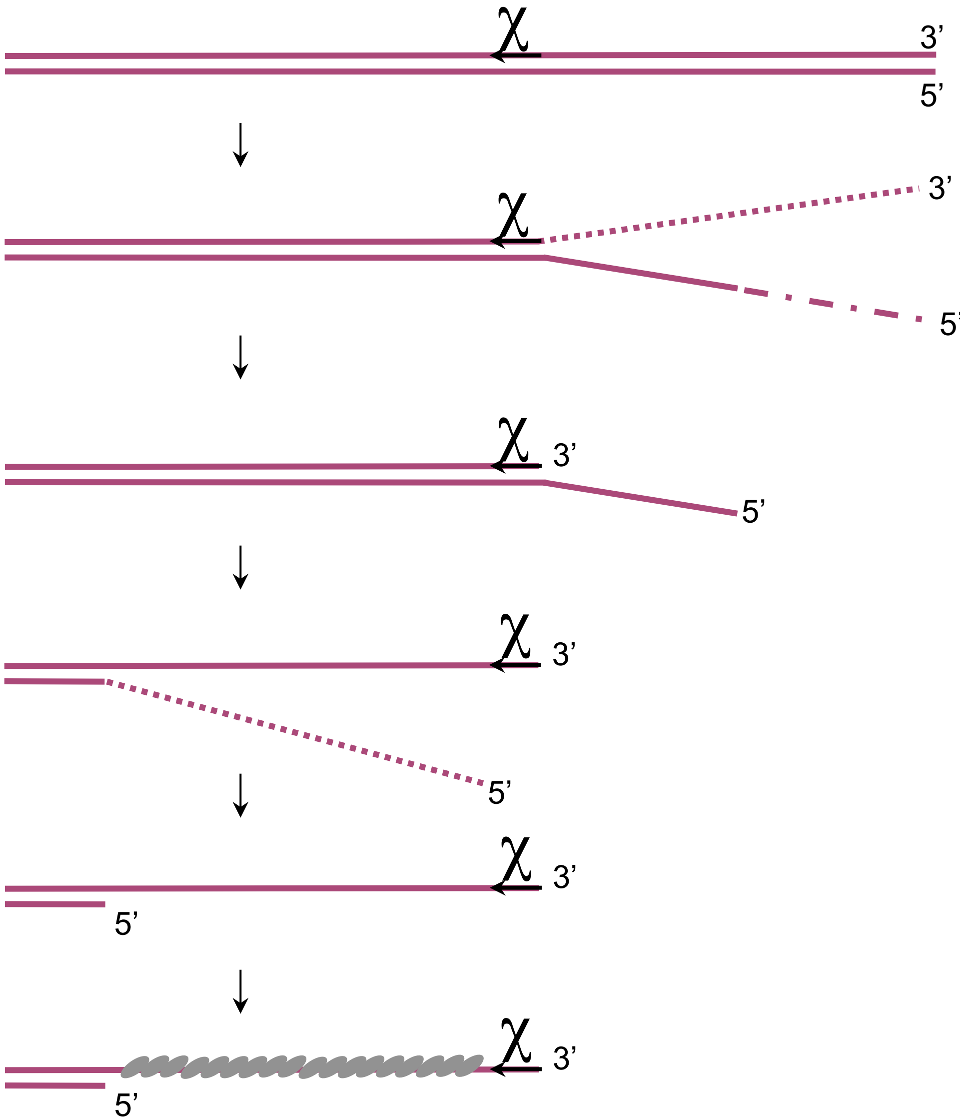
Pathways of Double-Strand Break Repair:

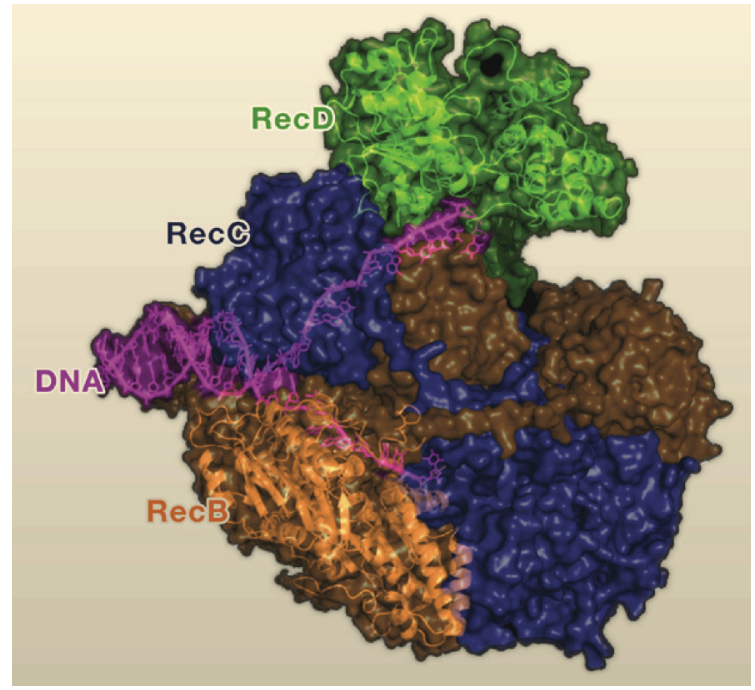


Processing of DNA ends for homologous recombination in eukaryotes:



RecBCD-mediated processing of DNA breaks in bacteria:

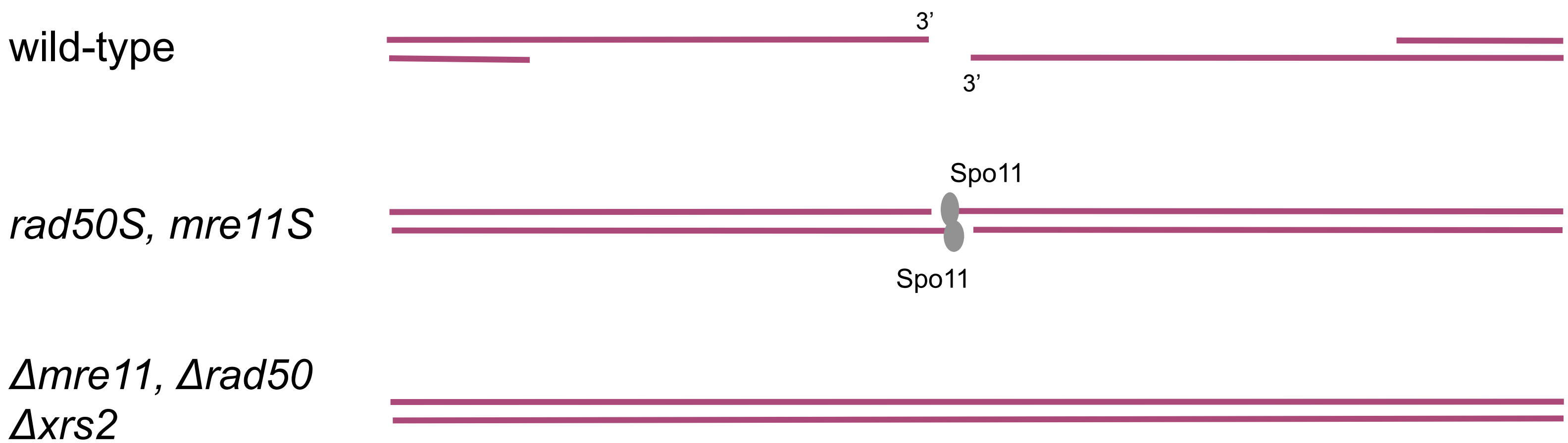




Singleton, M.R., Dillingham, M.S., Gaudier M., Kowalczykowski, S.C., and Wigley, D.B. (2004) Nature 432: 187-193.

but orthologs of RecBCD not apparent in archaea or eukaryotes

Phenotypes of *rad50* and *mre11* mutants during meiosis:

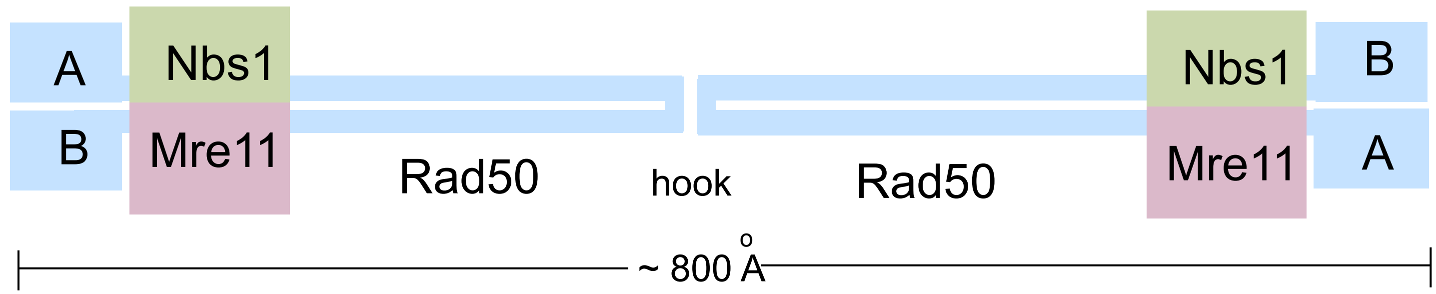


Mre11 and Rad50 inferred to play a role in resection based on resection defects during meiosis

Alani, E., Padmore, R., and Kleckner, N. (1990). Analysis of wild-type and rad50 mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell 61, 419-436.

Nairz, K., and Klein, F. (1997). mre11S--a yeast mutation that blocks double-strand-break processing and permits nonhomologous synapsis in meiosis. Genes & Dev. 11, 2272-2290.

Mre11/Rad50/Nbs1(Xrs2)

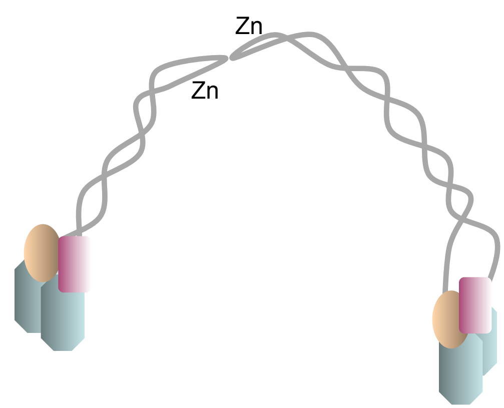


Mre11:
3' to 5' exonuclease and endonuclease on hairpin loops and ss/ds transitions

Rad50: ATPase in the ABC transporter family; similar to the Structural Maintenance of Chromosomes (SMC) family of chromosome condensation and chromatid cohesion proteins

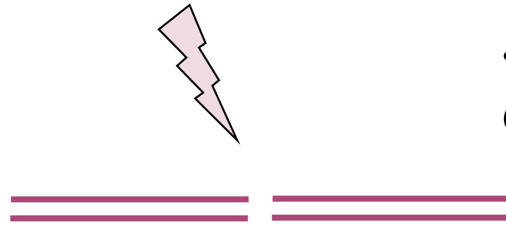
Nbs1 (mammals) or Xrs2 (yeast): regulatory component, only found in eukaryotes, controls both Mre11 and Rad50 enzymatic activities

zinc-mediated hook associations mediate intermolecular connections between Rad50 molecules:

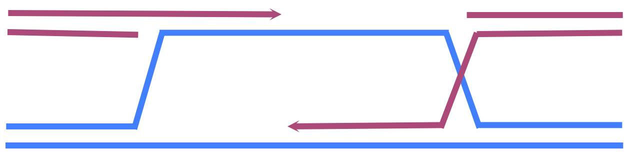


Functions of the Mre11/Rad50/Xrs2 complex in *S. cerevisiae*:

➜ required for survival of DNA double-strand breaks



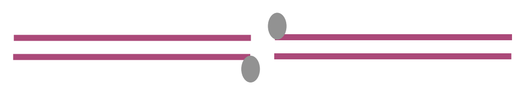
➜ required for S-phase checkpoint response



homologous recombination

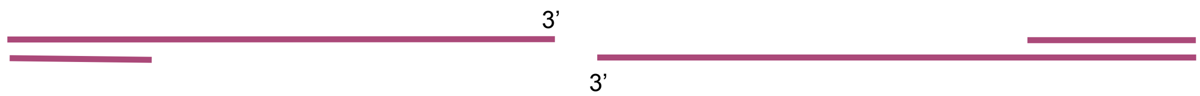
non-homologous end joining (NHEJ)

➜ telomere maintenance



➜ meiotic recombination

The polarity paradox:



DNA ends are resected from 5’ to 3’ but...

• The direction of Mre11 exonuclease activity in vitro is 3’ to 5’

• resection of DNA ends in vegetatively growing cells is not dependent on Mre11 nuclease activity (even though meiotic DSB resection is Mre11 nuclease-dependent).

Yet...

• overexpression of ExoI (5’ to 3’ exonuclease) can suppress DNA repair defects seen in MRX-deficient strains

Paull, T.T., and Gellert, M. (1998). Mol. Cell 1, 969-979.

Moreau, S., Ferguson, J.R., and Symington, L.S. (1999). Mol. Cell. Biol. 19, 556-566.

Moreau, S., Morgan, E.A., and Symington, L.S. (2001). Genetics 159, 1423-1433.

Ben Hopkins

The Mre11 and Rad50 genes cluster with a helicase (HerA) and a nuclease (NurA) in thermophilic archaea:

? Do Mre11, Rad50, HerA, and NurA form a multisubunit enzyme for DNA end processing ?

Pyrococcus furiosus: thermophilic archaeon, grows at high temperatures (75 - 100˚C)

- HerA from *Sulfolobus* and *Pyrococcus* species exhibits both 5’ and 3’ helicase activities in vitro
- NurA resects linear dsDNA from 5’ to 3’ and also exhibits endonuclease activity on ssDNA

F. Constantinesco, P. Forterre, and C. Elie (2002) EMBO Rep. 3: 537-542.

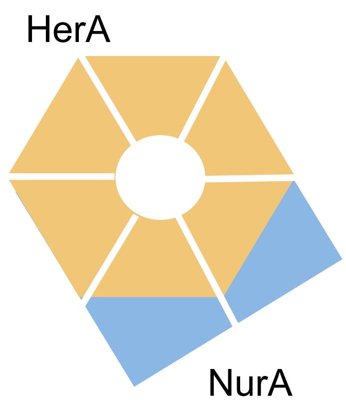
F. Constantinesco, P. Forterre, E.V. Koonin, L. Aravind, and C. Elie (2004) NAR 32: 1439-1447.

A. Manzan, G. Pfeiffer, M.L. Hefferin, C.E. Lang, J.P. Carney, and K.P. Hopfner (2004) EMBO Rep. 5: 54-59.

S. Zhang, T. Wei, G. Hou, C. Zhang, P. Liang, J. Ni, D. Sheng, and Y. Shen (2008) DNA Repair 7: 380-391.

Recombinant pfHerA (helicase) and pfNurA (nuclease) are functionally interdependent:

- pfHerA and pfNurA physically interact in the absence of DNA or ATP
- no detectable interactions between pfHerA/NurA and pfMre11/Rad50
- pfNurA is a Mn-dependent 5' – 3' exonuclease but shows activity in Mg in the presence of pfHerA
- pfHerA helicase activity is stimulated ~3 to 5-fold by pfNurA



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Recombinant pfMre11/Rad50 stimulates resection of linear DNA ends by pfHerA/NurA by ~20-fold:

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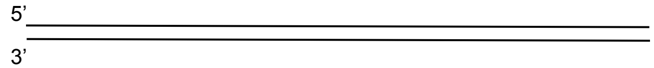
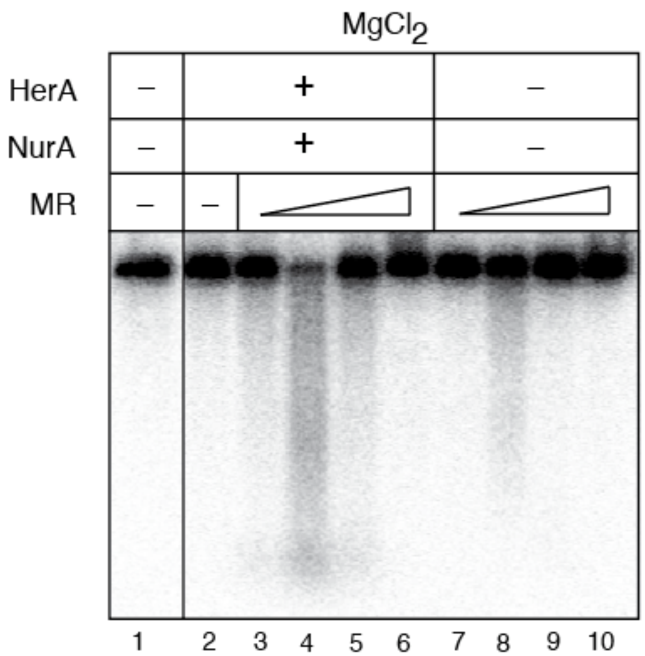
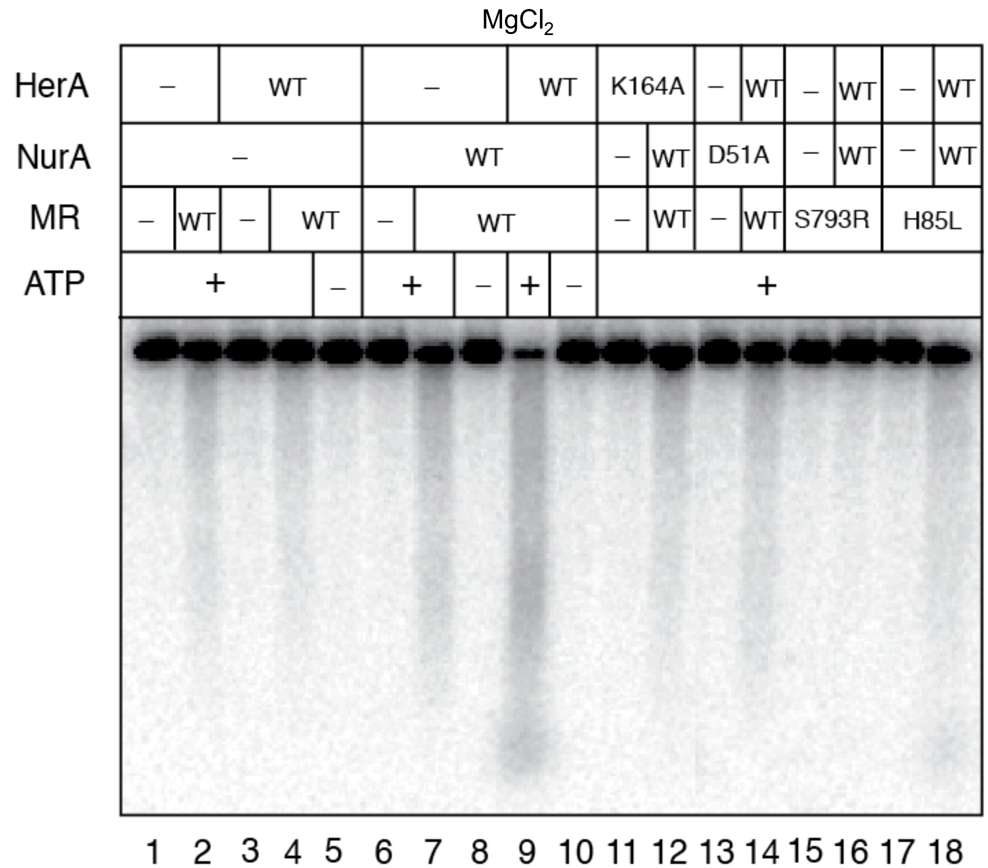


2.5 Kb internally linear DNA substrate

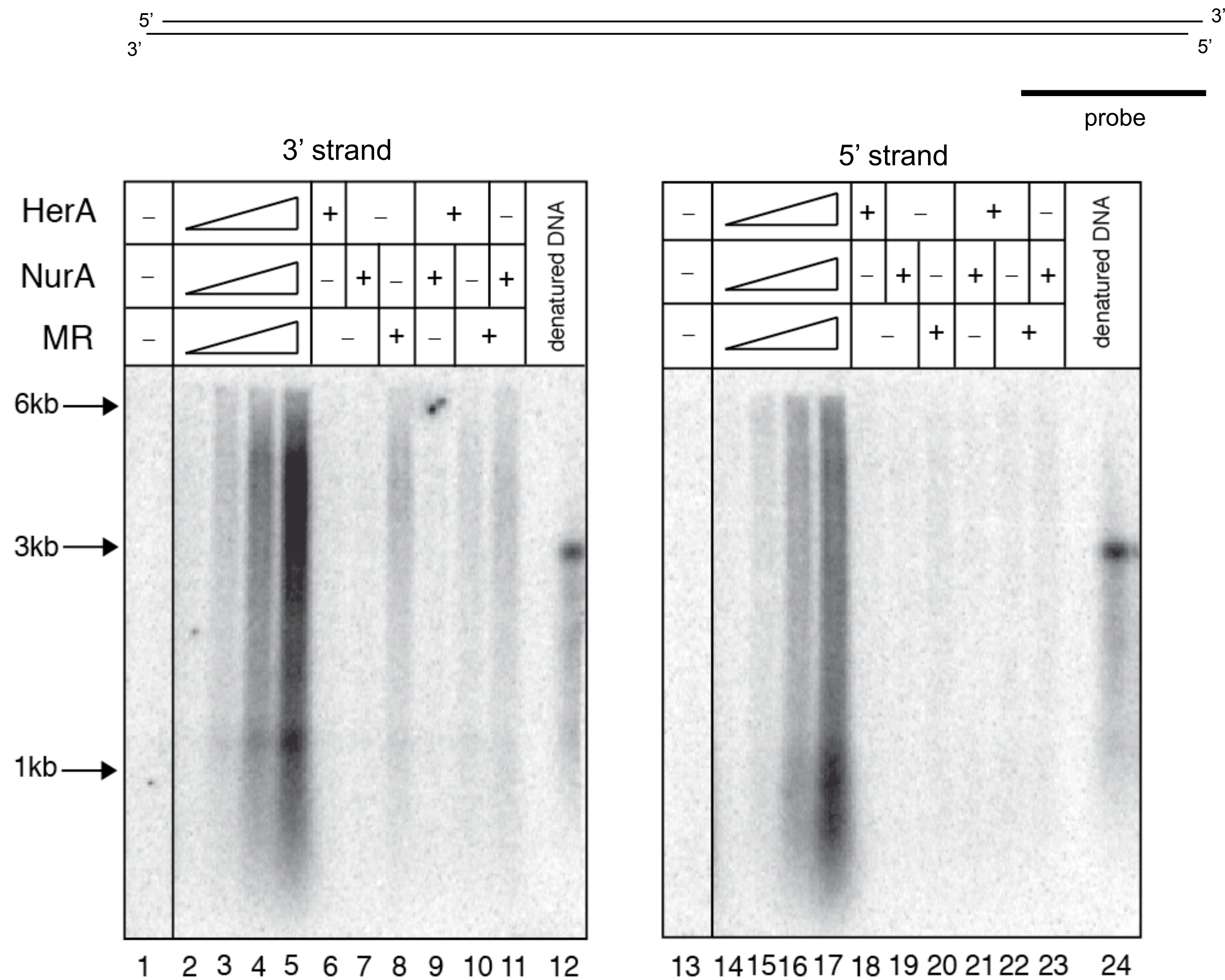


20 nM NurA (monomer)
2.7 nM HerA (hexamer)
0.3, **3.3**, 33, 330 nM Mre11/Rad50 (M2R2)
1 hr, 65°C



20 nM NurA (monomer)
2.7 nM HerA (hexamer)
3.3 nM Mre11/Rad50 (M2R2)
1 hr, 65°C

Strand-specific probes show resection of both strands, but more pronounced resection of the 5' strand:



5, 10, 20, 40 nM NurA (monomer); 0.7, 1.4, 2.7, 5.4 nM HerA (hexamer); 0.8, 1.6, 3.3, 6.6 nM Mre11/Rad50 (M2R2), 5 mM $MgCl_2$, 1 mM ATP, 15 min, 65°C

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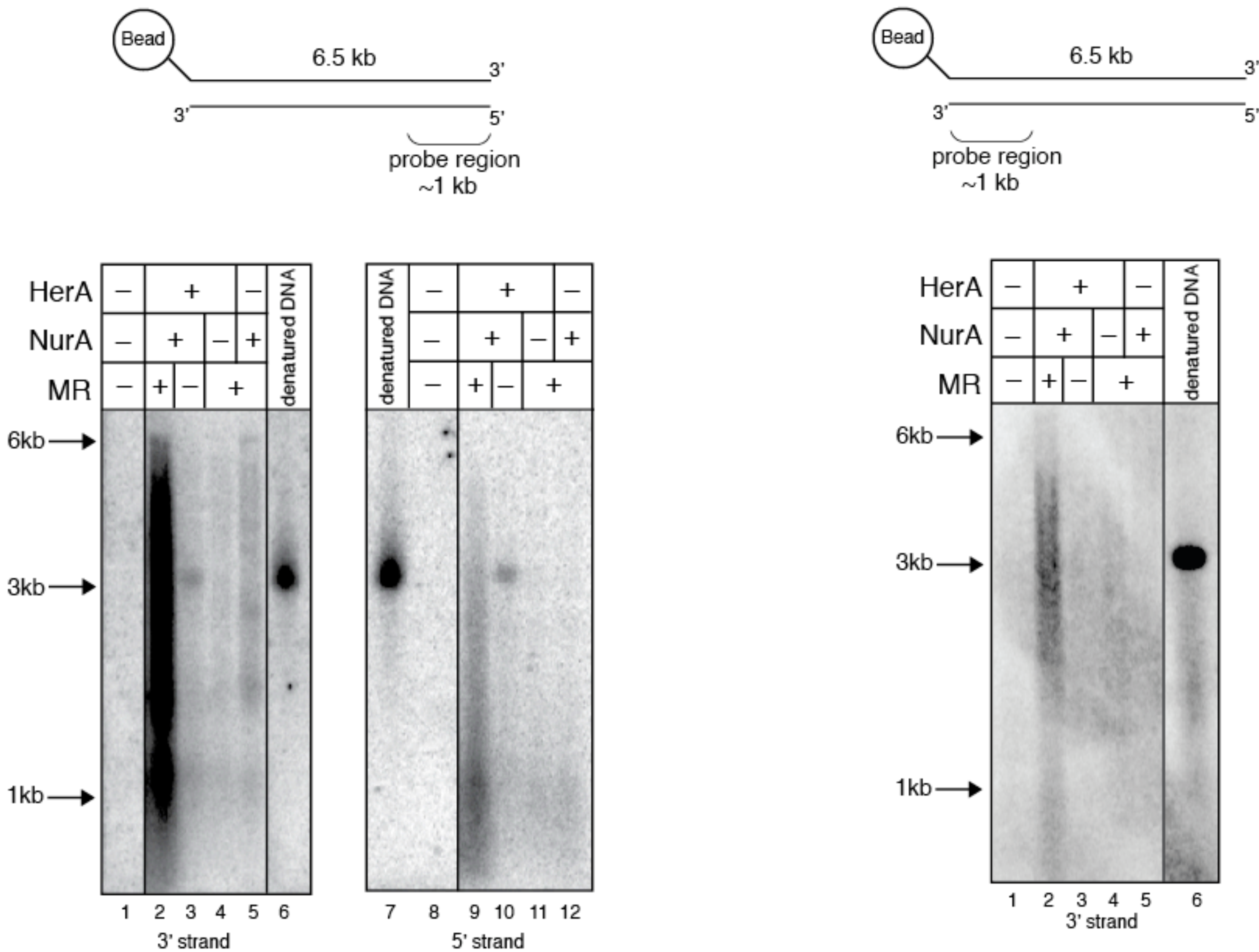
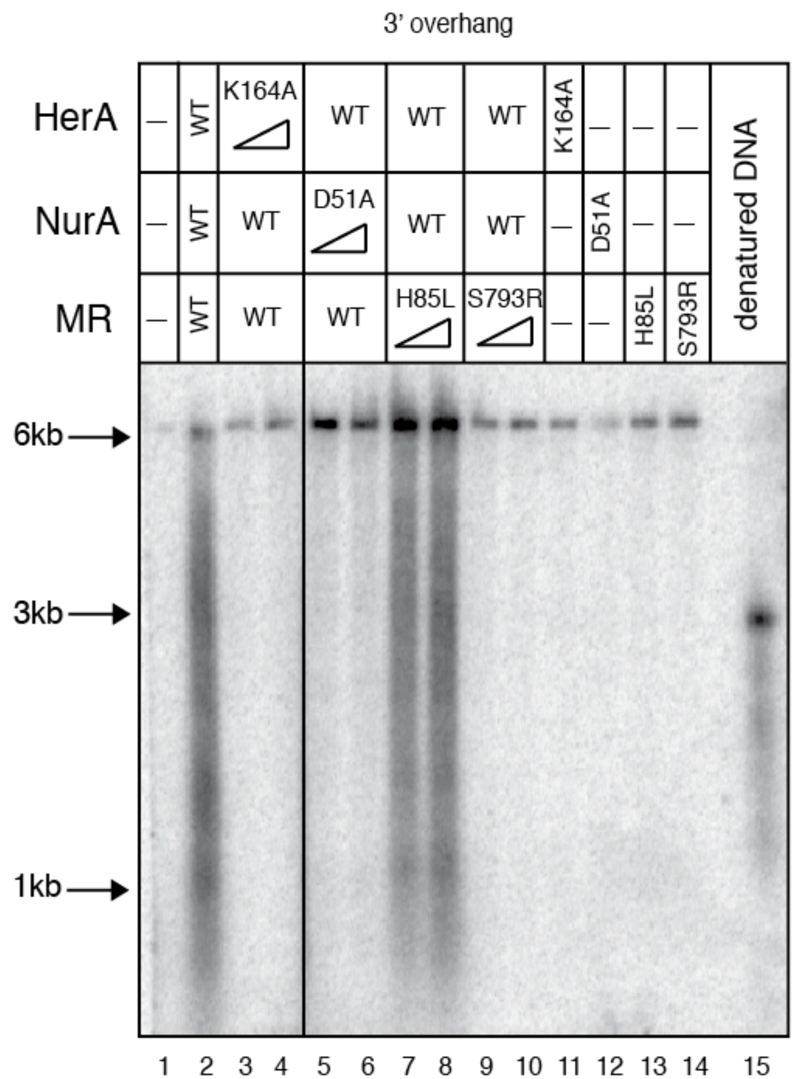
The direction of pfMRHN-catalyzed resection is 5' to 3':



40 nM NurA (monomer); 5.4 nM HerA (hexamer); 6.6 nM Mre11/Rad50 (M2R2)
5 mM $MgCl_2$, 1 mM ATP, 65°C

Cooperative DSB resection requires catalytic activities of pfRad50, pfHerA, and pfNurA, but not pfMre11:



20, 40 nM NurA (monomer); 2.7, 5.4 nM HerA (hexamer); 3.3, 6.6 nM Mre11/Rad50 (M2R2)
5 mM $MgCl_2$, 1 mM ATP, 15 min, 65°C

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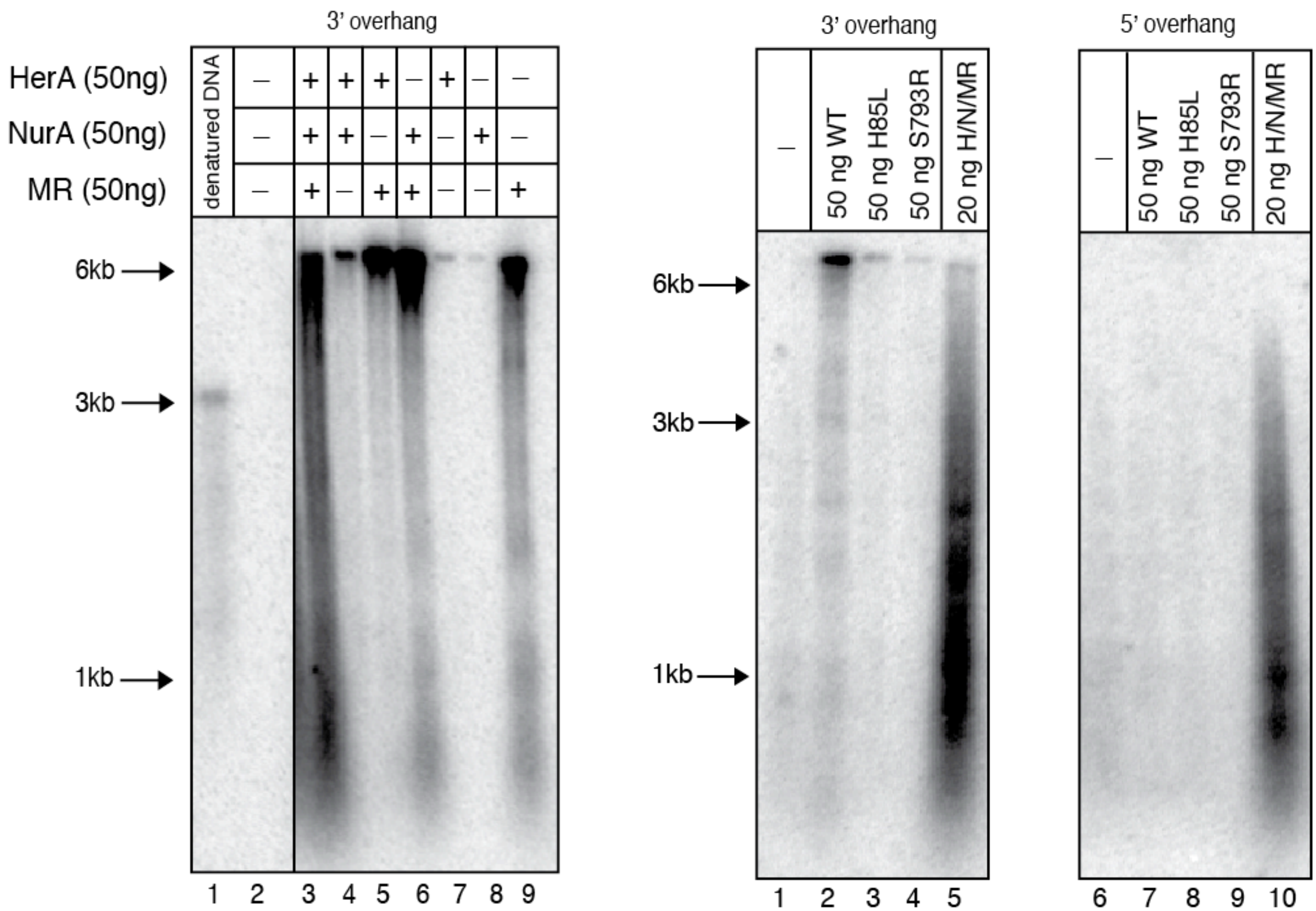
pfMre11/Rad50 catalyzes short-range resection of the 5' strand of a DSB, dependent on the nuclease activity of pfMre11:



50 ng = 100 nM NurA (monomer); 13.5 nM HerA (hexamer); 16.5 nM Mre11/Rad50 (M2R2)
5 mM $MgCl_2$, 1 mM ATP, 15 min, 65°C

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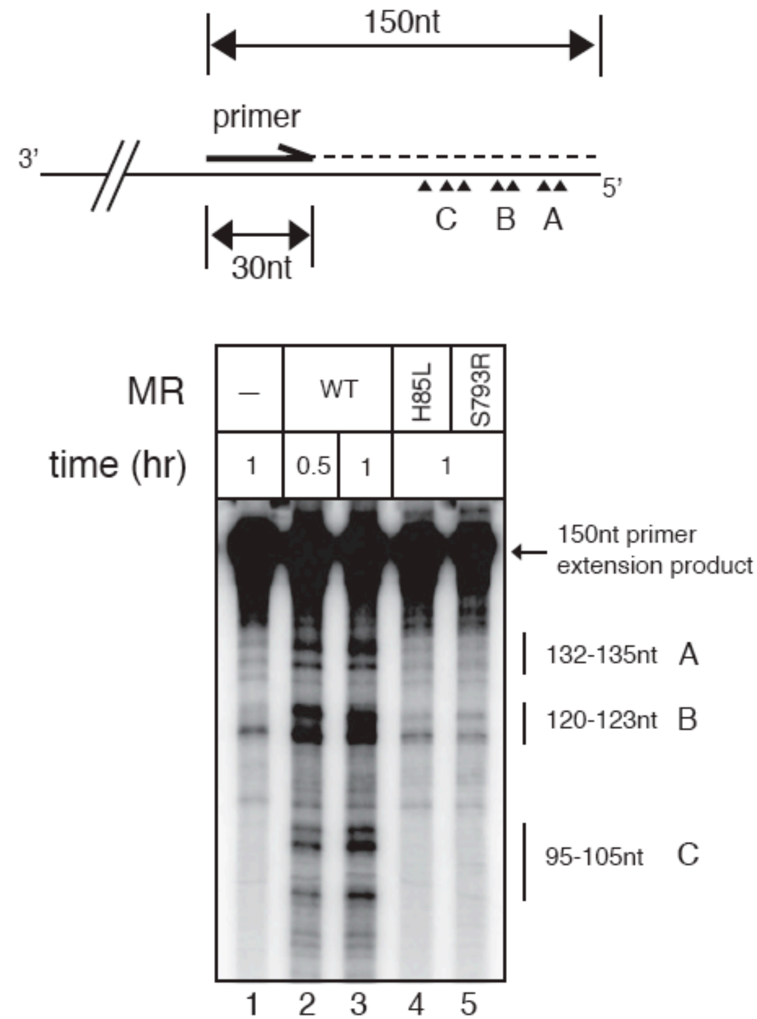
pfMre11/Rad50 does exhibit nuclease activity in $MgCl_2$ at physiological temperature:

endonucleolytic cleavage events on the 5' strand



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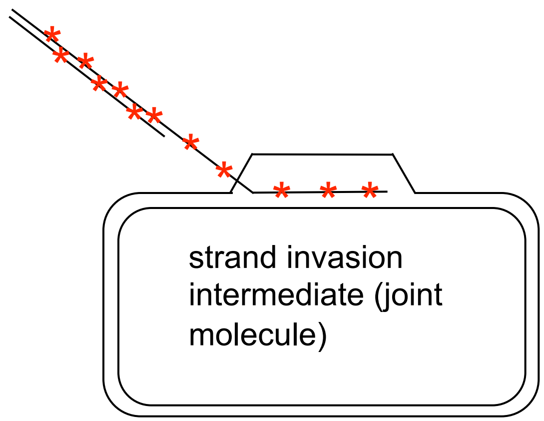
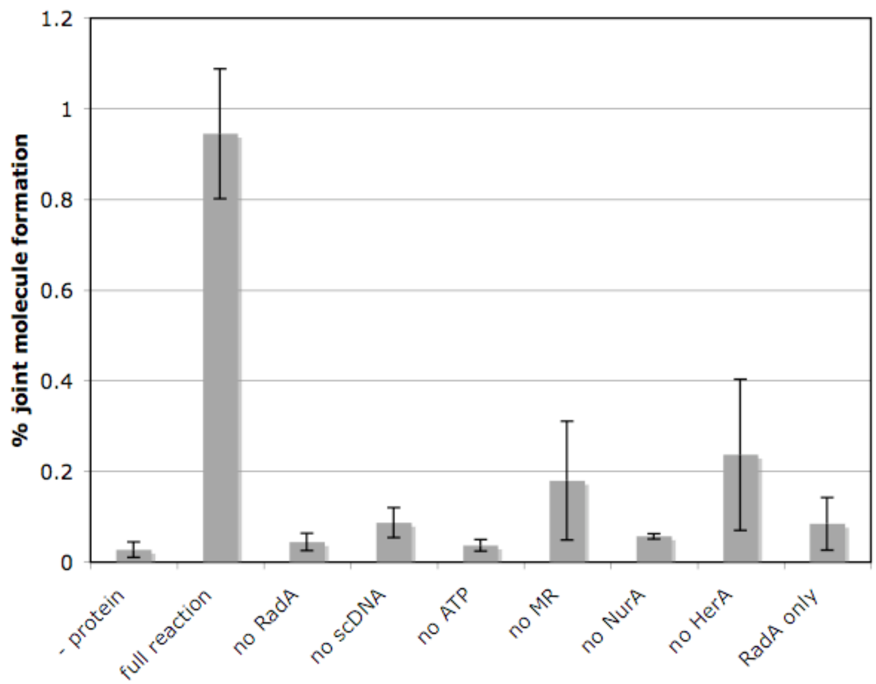
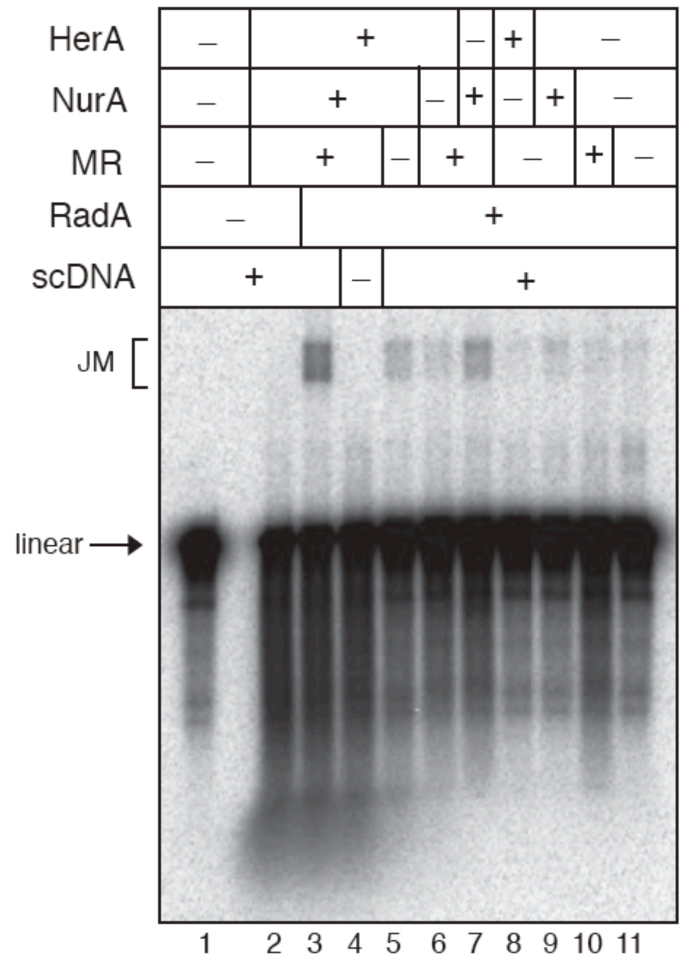
pfMRHN function cooperatively with pfRadA in strand invasion:

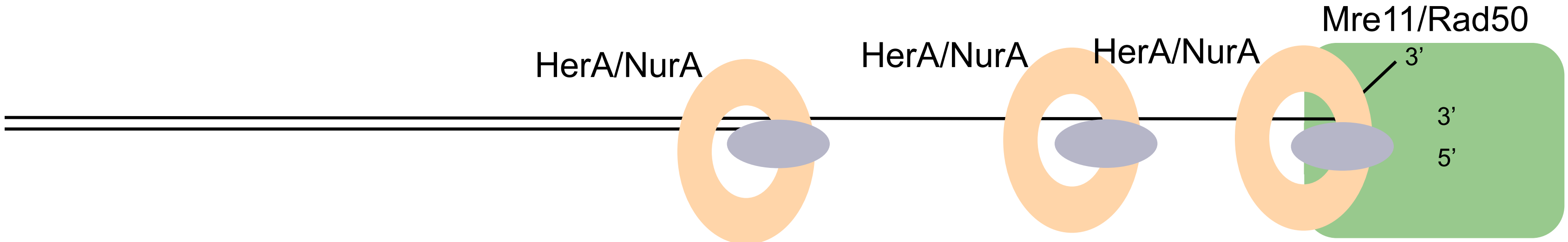






200 nM NurA (monomer); 27 nM HerA (hexamer); 33 nM Mre11/Rad50 (M2R2); 50 nM RadA; 10 mM $MgCl_2$, 2 mM ATP, 0.2 nM linear DNA, 2 nM scDNA; 30 min, 55°C

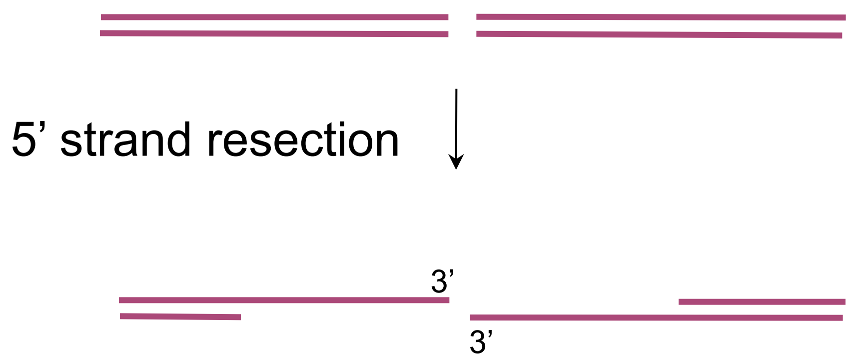
Working model for DSB processing by pfMre11/Rad50/HerA/NurA:



Double-strand break processing in eukaryotes:

Factors implicated in DNA end processing in *S. cerevisiae:*

- Mre11/Rad50/Xrs2 (MRX)
- Sae2
- Exo1
- Sgs1(Rmi1/Top3)/Dna2



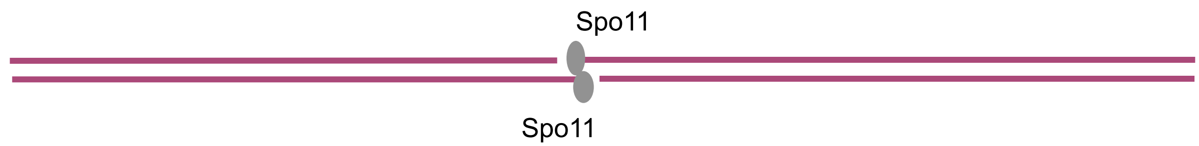
Removal of any single complex results in a delay of resection in vivo;
resection completely blocked by removal of MRX/Sae2 + Exo1 + Sgs1/Dna2

Mimitou, E.P. and Symington, L.S. (2008) Nature 455: 770-774.

Zhu and Ira, G. (2008) Cell 134:981-994

Gravel, S., Chapman, J.R., Magill, C., and Jackson, S. P. (2008) Genes & Dev. 22:2767-2772.

Sae2 and the Mre11/Rad50/Xrs2 (MRX) complex in *S. cerevisiae*:



- Sae2 is required for the processing of double-strand breaks made by Spo11 during prophase of meiosis I; phenotype similar to that of Rad50S (covalent, unprocessed Spo11-DNA conjugates)

McKee, A. H., and Kleckner, N. (1997) Genetics *146*, 797-816.

Prinz, S., Amon, A., and Klein, F. (1997) Genetics *146*, 781-795.

- Sae2 also is important for chromosomal DNA repair in vegetative cells (assay for intrachromosomal homologous recombination)

Rattray, A. J., McGill, C. B., Shafer, B. K., and Strathern, J. N. (2001). Genetics *158*, 109-122.

- Sae2 (and MRX) are essential for the processing of hairpin-capped double-strand breaks

Lobachev, K. S., Gordenin, D. A., and Resnick, M. A. (2002). Cell *108*, 183-193.

Rattray, A. J., Shafer, B. K., Neelam, B., and Strathern, J. N. (2005). Genes Dev *19*, 1390-1399.

Sae2 phosphorylation and effects on DSB processing:

• Sae2 is phosphorylated on several SQ/TQ sites during S/G_2 and after DNA damage

S. cerevisiae Sae2:

Baroni, E., Viscardi, V., Cartagena-Lirola, H., Lucchini, G., and Longhese, M. P. (2004). Mol Cell Biol *24*, 4151-4165.

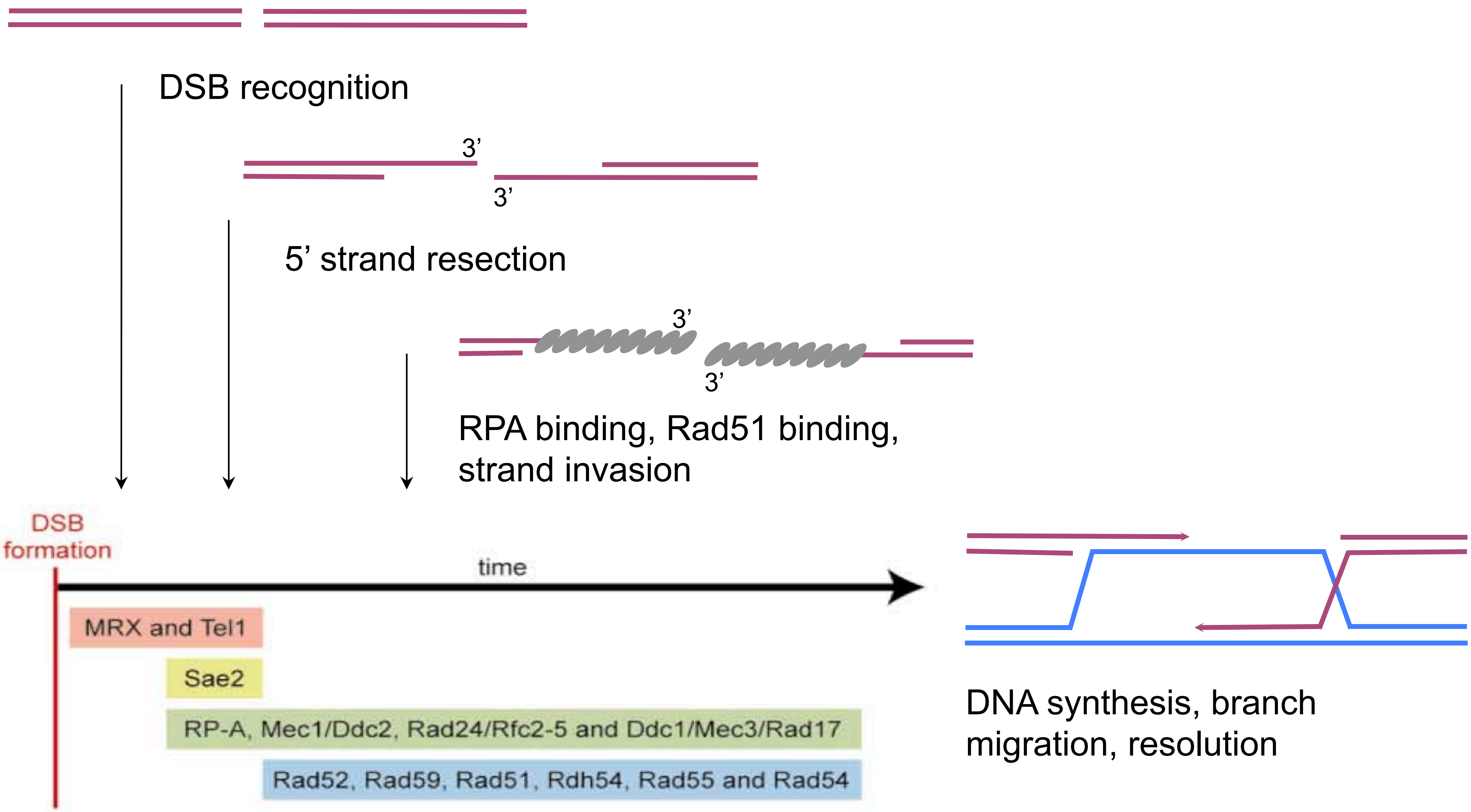
• In the absence of Sae2 (or ΔMRX or MRX(Rad50S)), processing of DSBs into 3' single-strands is delayed and the efficiency of single-strand annealing is significantly reduced

Clerici, M., Mantiero, D., Lucchini, G., and Longhese, M. P. (2005). J Biol Chem *280*, 38631-38638.

• Sae2 is phosphorylated in S/G_2 by CDK on S267; mutation of this serine to the phosphomimic glutamate can drive resection in G_1 phase

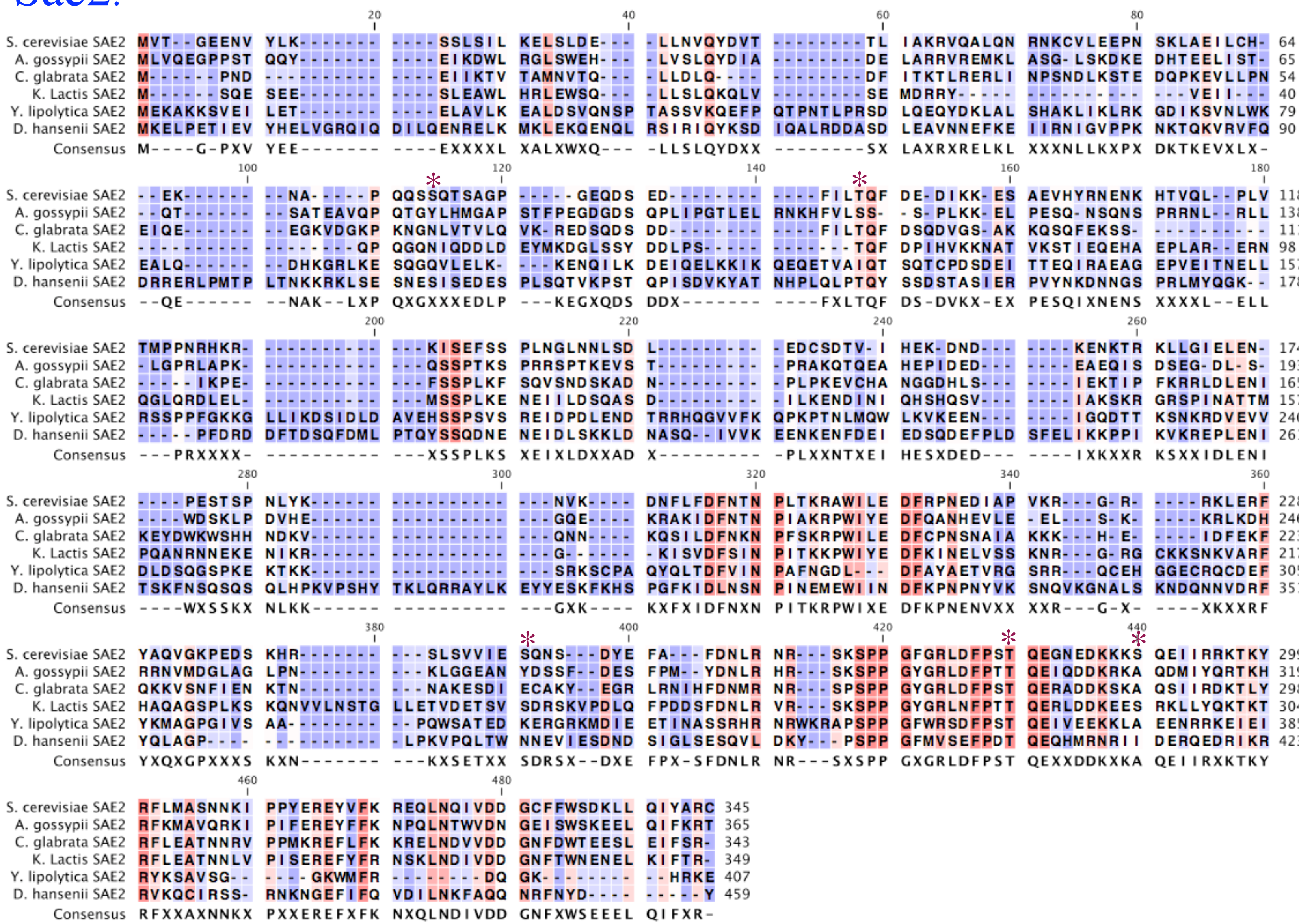
Huertas, P., F. Cortes-Ledesma, A.A. Sartori, A. Aguilera, and S.P. Jackson. 2008. CDK targets Sae2 to control DNA-end resection and homologous recombination. Nature 455: 689-692.

Microscopy and chromatin immunoprecipitation assays show that MRX and Sae2 localize to DNA breaks first, then dissociate with Rad51 loading:



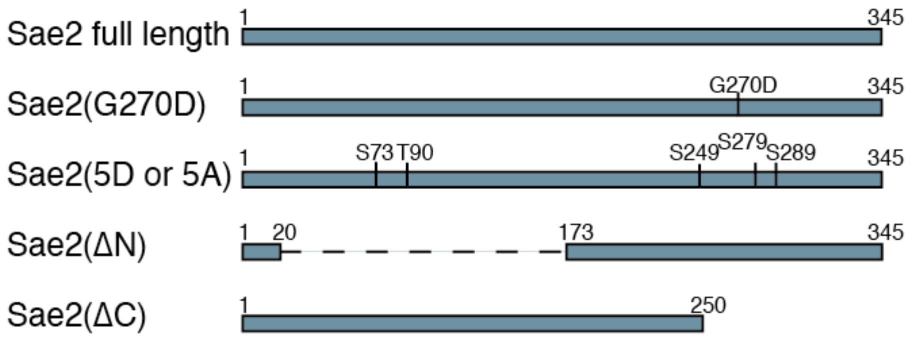
Lisby M, Barlow JH, Burgess RC, Rothstein R (2004) Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. Cell 118(6): 699-713

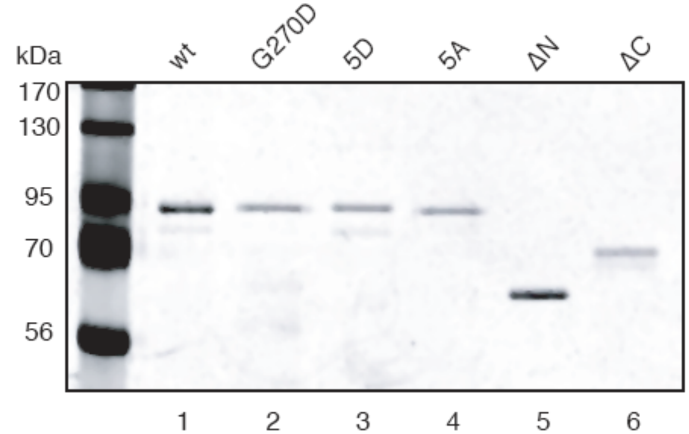
Sae2:



Sae2 phosphorylation: Baroni, E., Viscardi, V., Cartagena-Lirola, H., Lucchini, G., and Longhese, M. P. (2004). Mol Cell Biol *24*, 4151-4165.

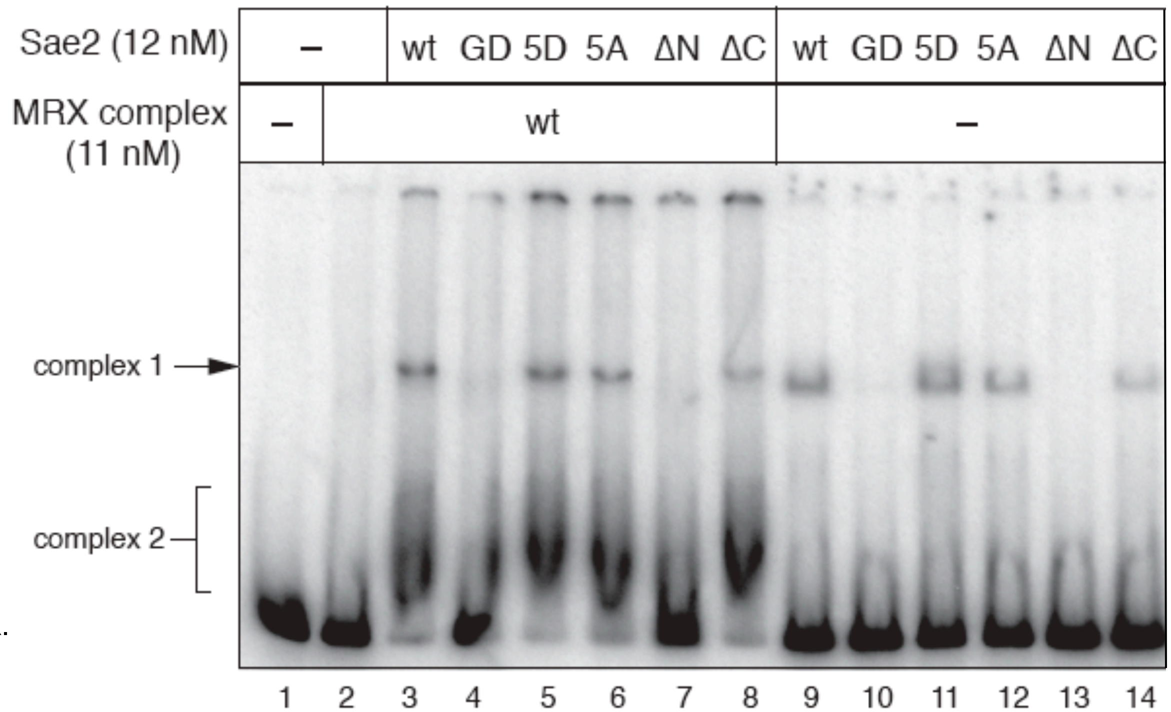
Recombinant Sae2:





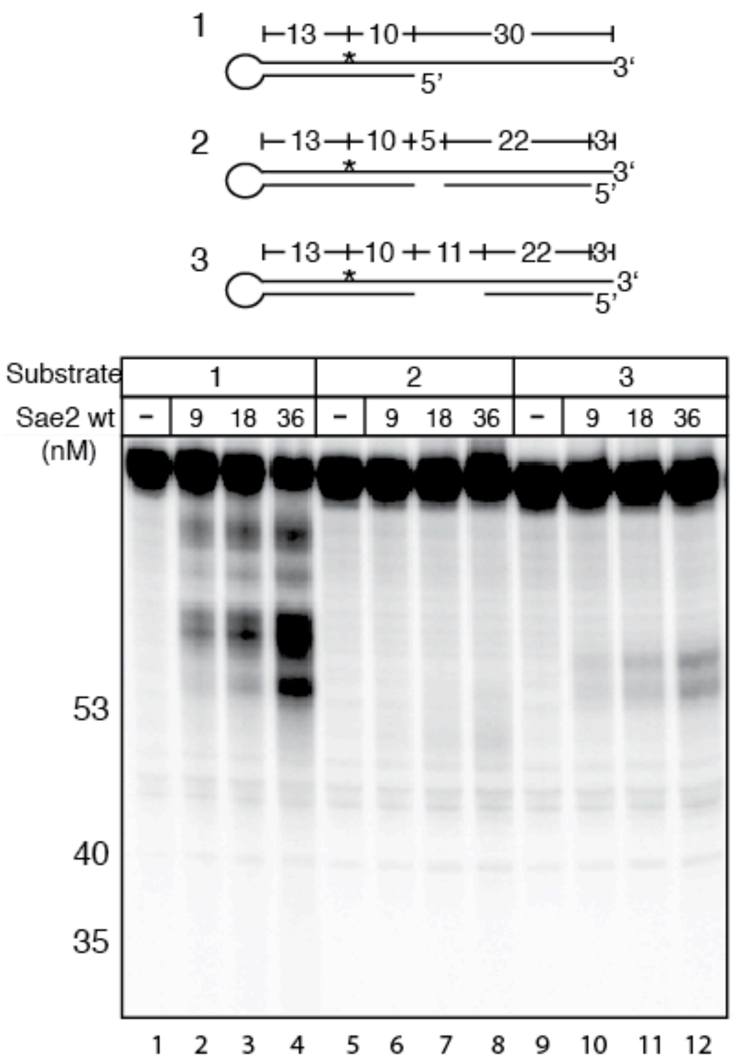
- dimeric and monomeric forms

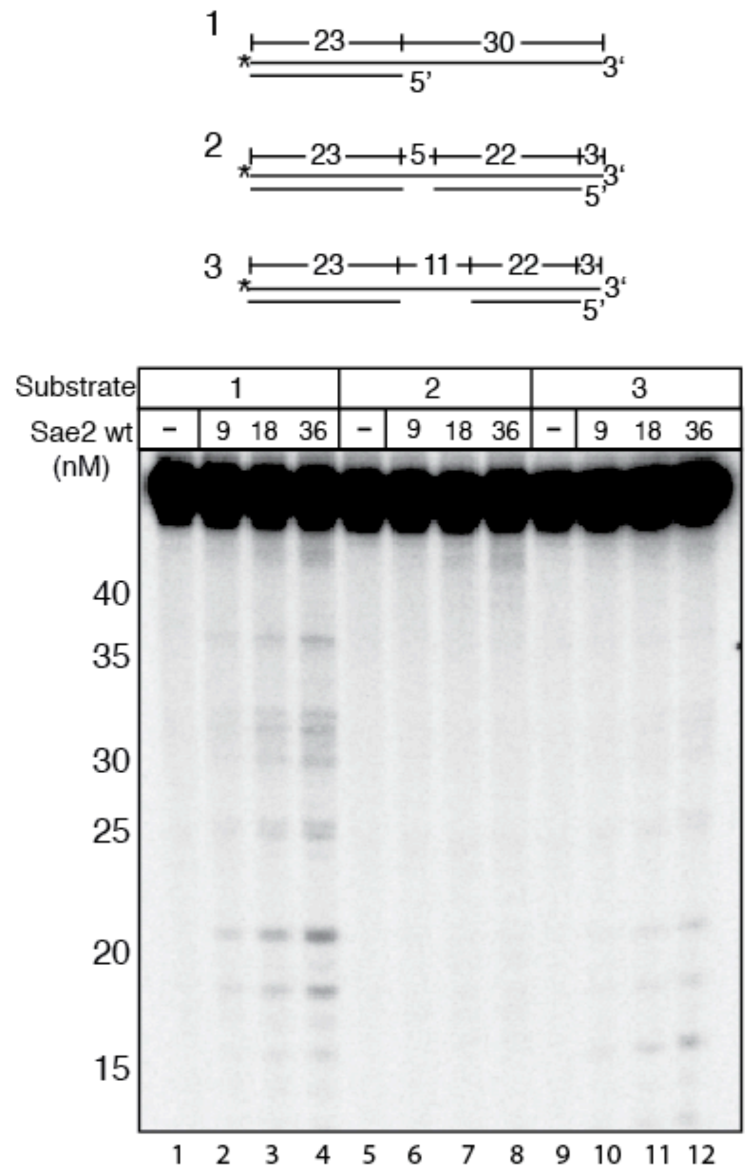
- binds linear DNA; forms complexes in response to MRX



Lengsfeld, B.M., A.J. Rattray, V. Bhaskara, R. Ghirlando, and T.T. Paull, (2007) Mol Cell, 28:638-51.

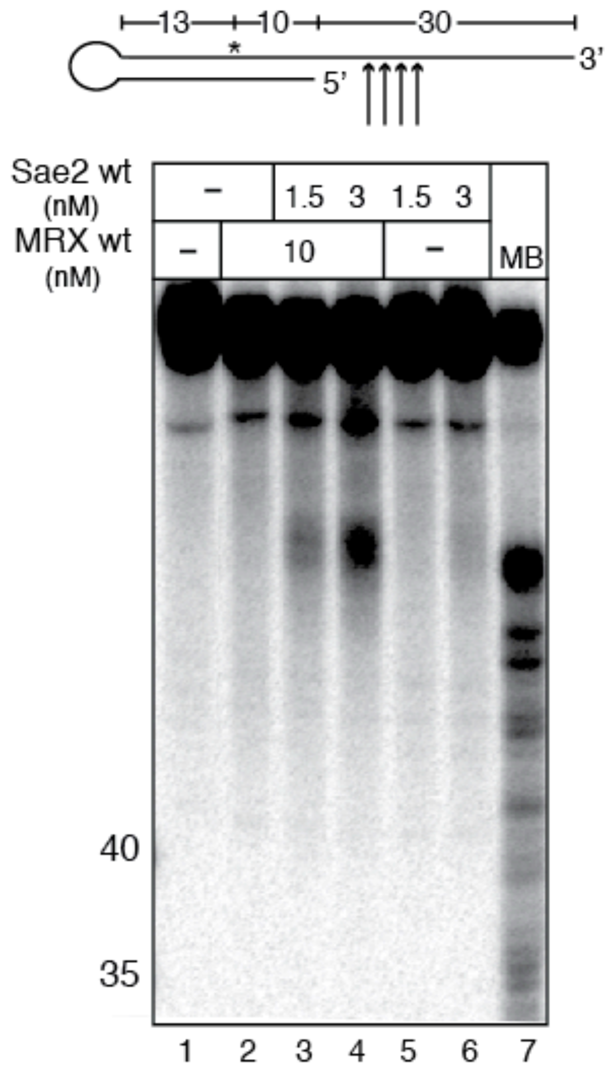
Sae2 exhibits endonuclease activity on hairpin DNA structures:

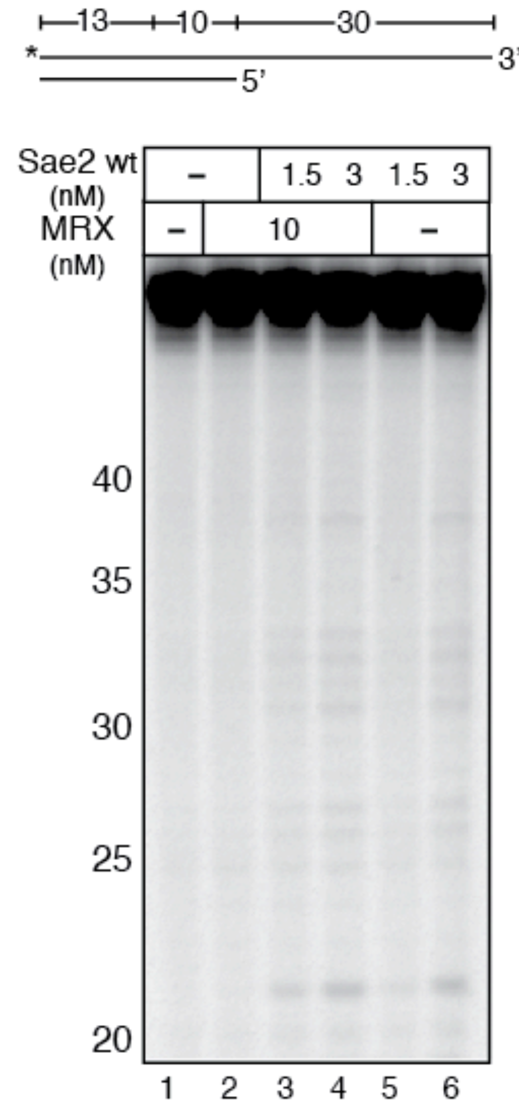




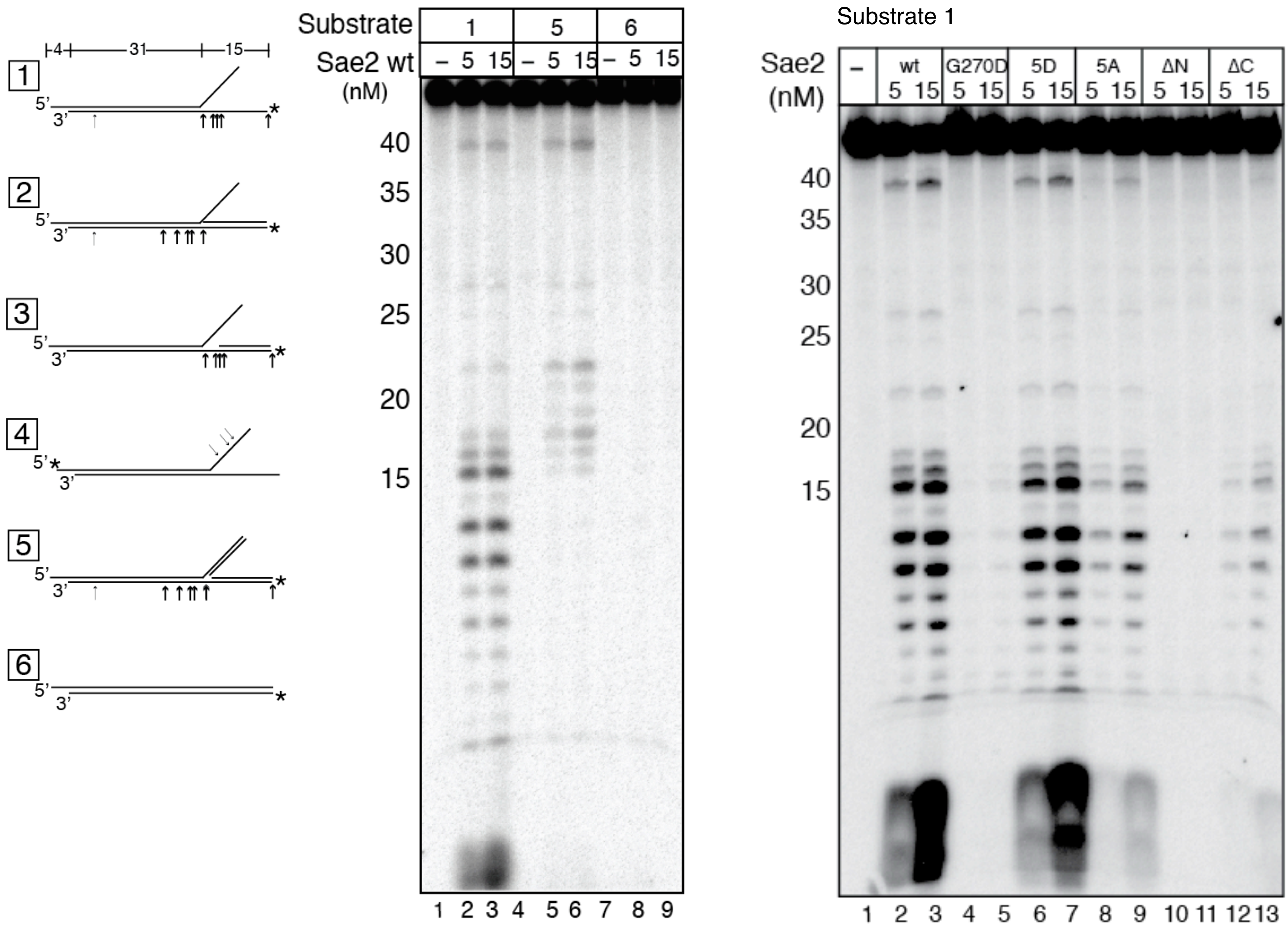
• but the cleavage sites are not at the hairpin tip

At lower levels of Sae2 and MRX, the complexes act cooperatively on hairpin structures:





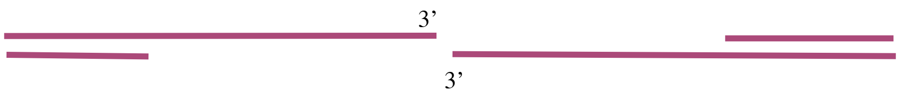
Sae2 exhibits MRX-independent flap endonuclease activity:



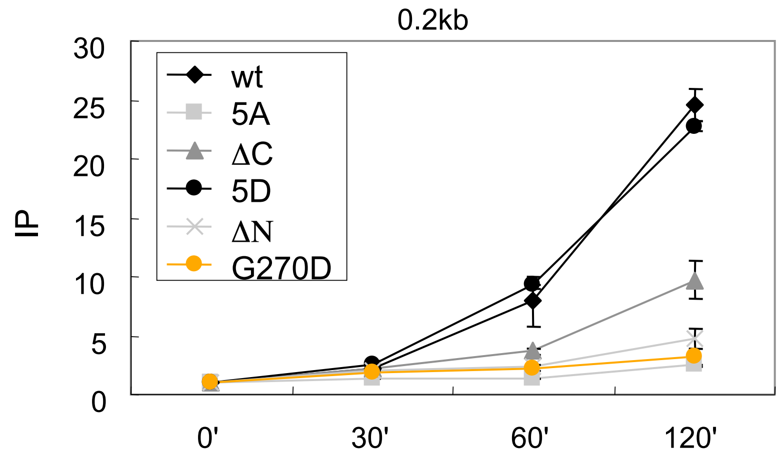
Sae2 mutant phenotypes in resection:

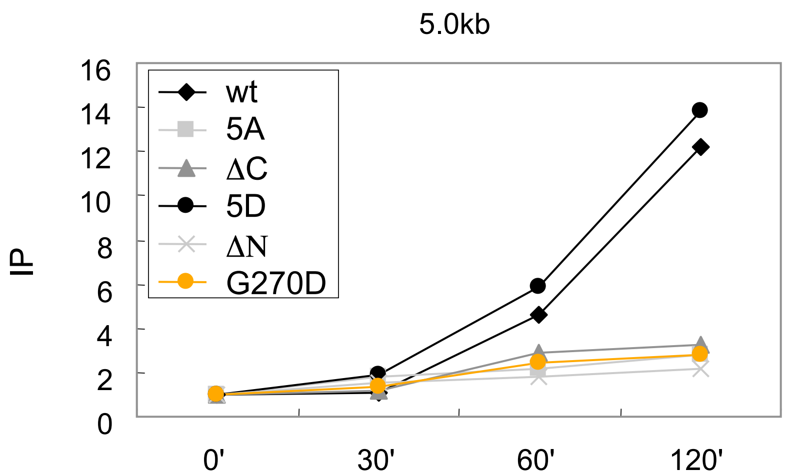
Sang Eun Lee
Kihoon Lee

Univ. of Texas Health Science Center,
San Antonio, TX

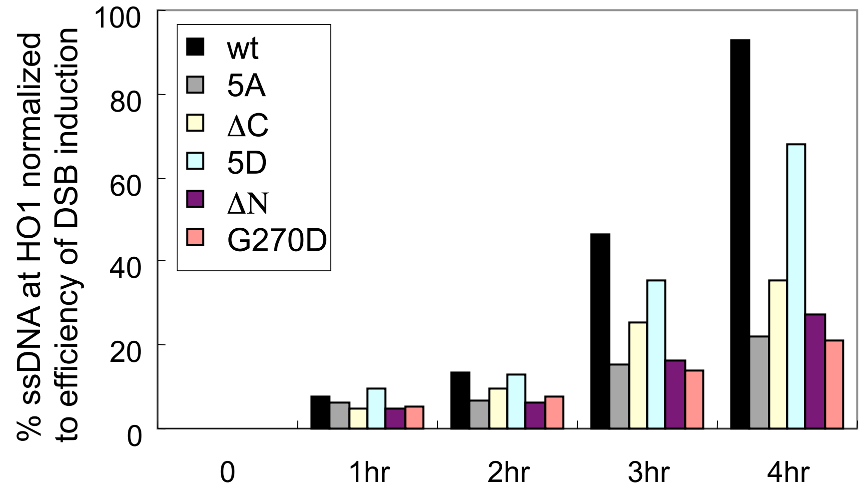


RPA binding to ssDNA (ChIP):



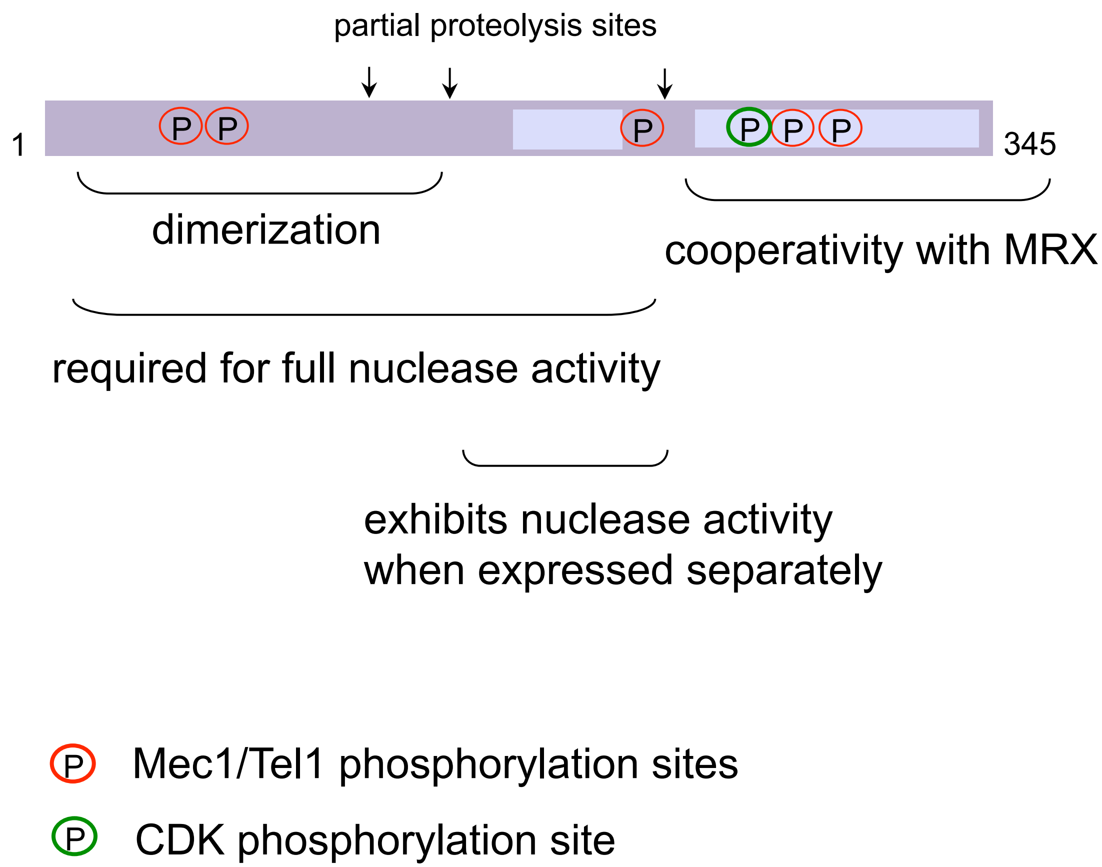


Resection:



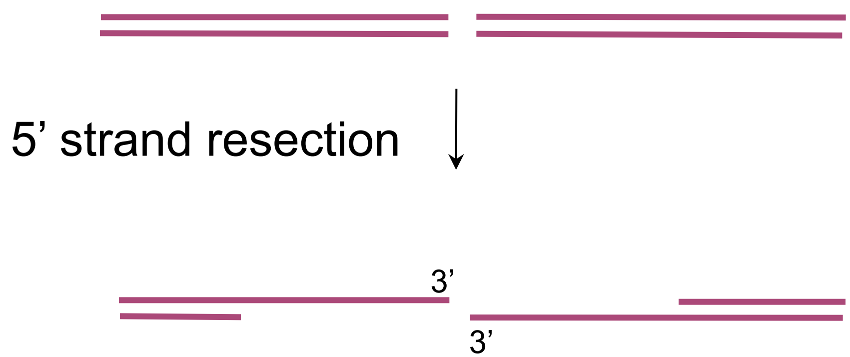
Mingjuan Shen

summary of Sae2 structure/function analysis:



Double-strand break processing in *S. cerevisiae*:

- Mre11/Rad50/Xrs2 (MRX)
- Sae2
- Exo1
- Sgs1(Rmi1/Top3)/Dna2

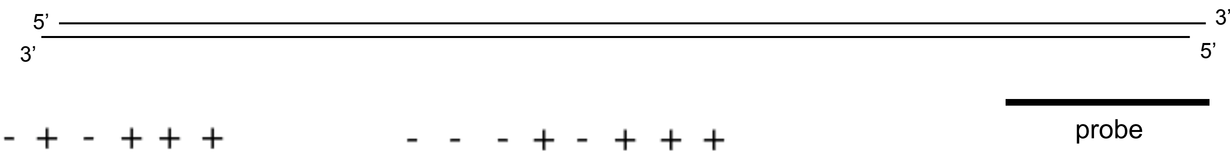


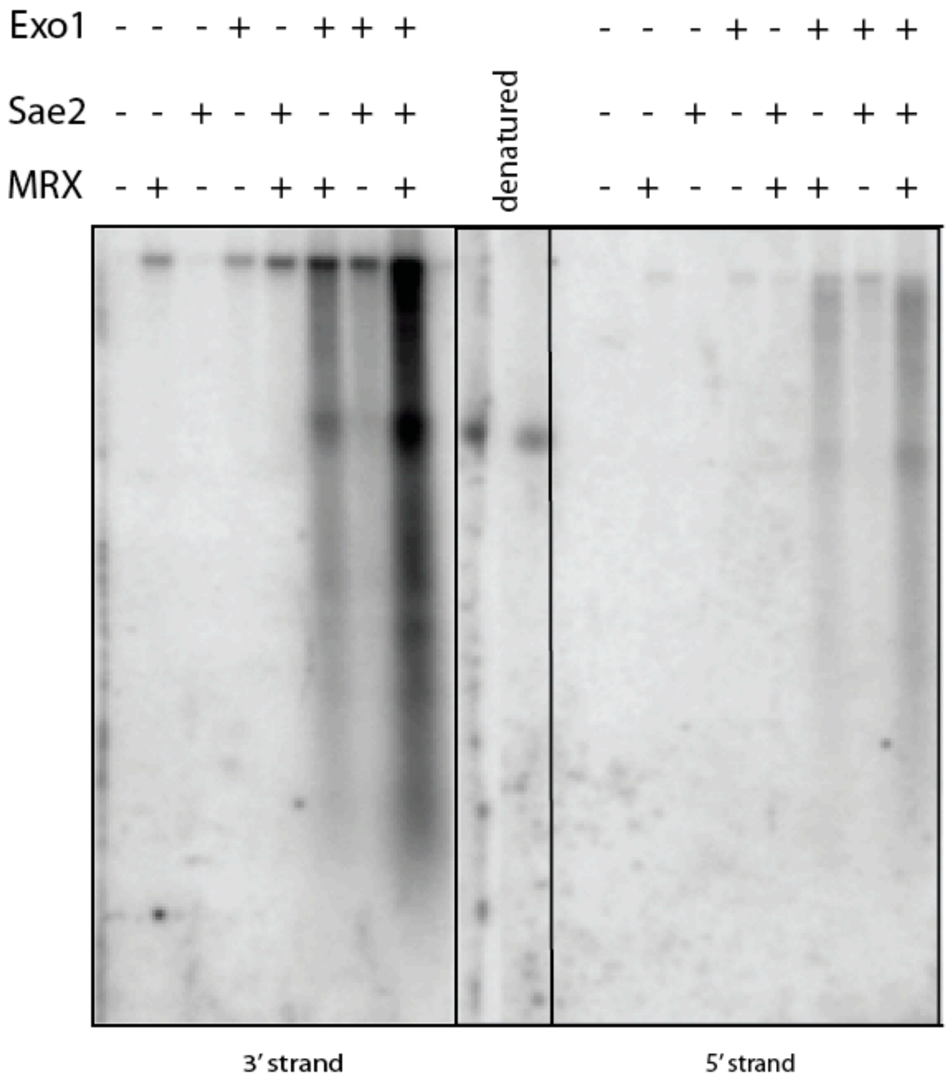
? Which factors are sufficient for 5' strand resection of DSB's?

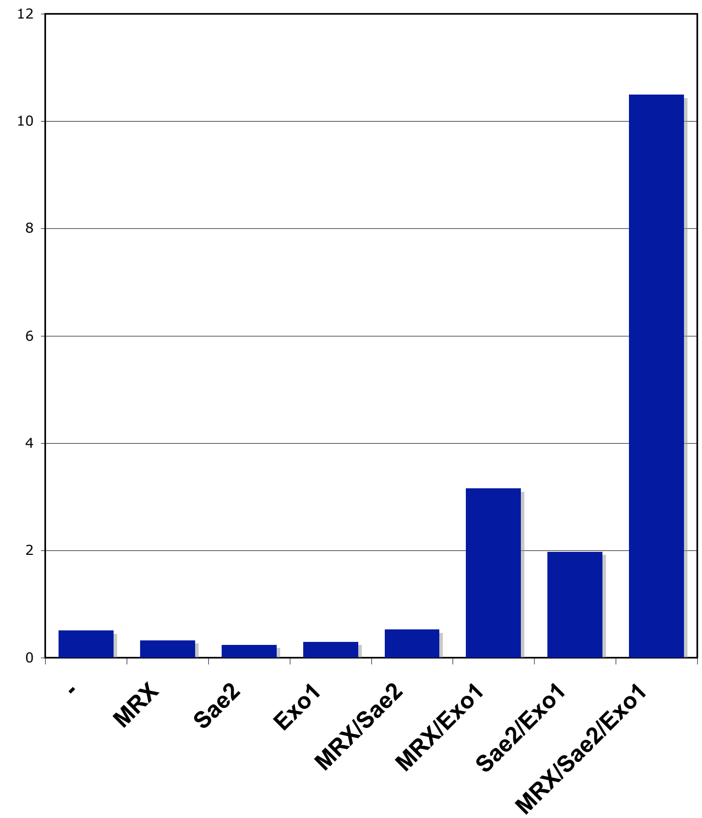
? Can we demonstrate this with purified components in vitro?

Recombinant yMRX and Sae2 function cooperatively with yExo1 in DNA end processing:

Matthew Nicolette



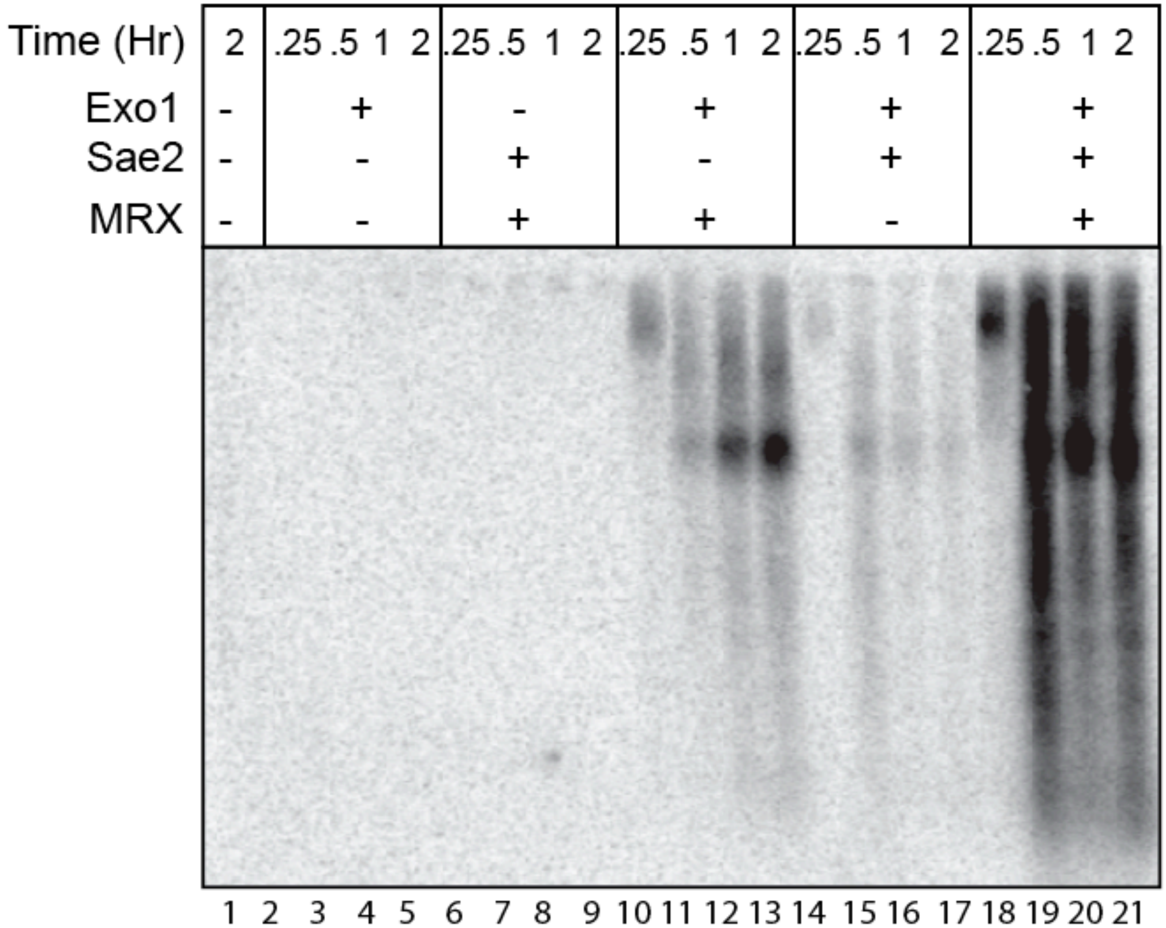




nondenaturing Southern blot with 6.5 kb plasmid DNA linearized substrate, strand-specific RNA probes

Matthew Nicolette

yExo1 acts cooperatively with yMRX and Sae2:



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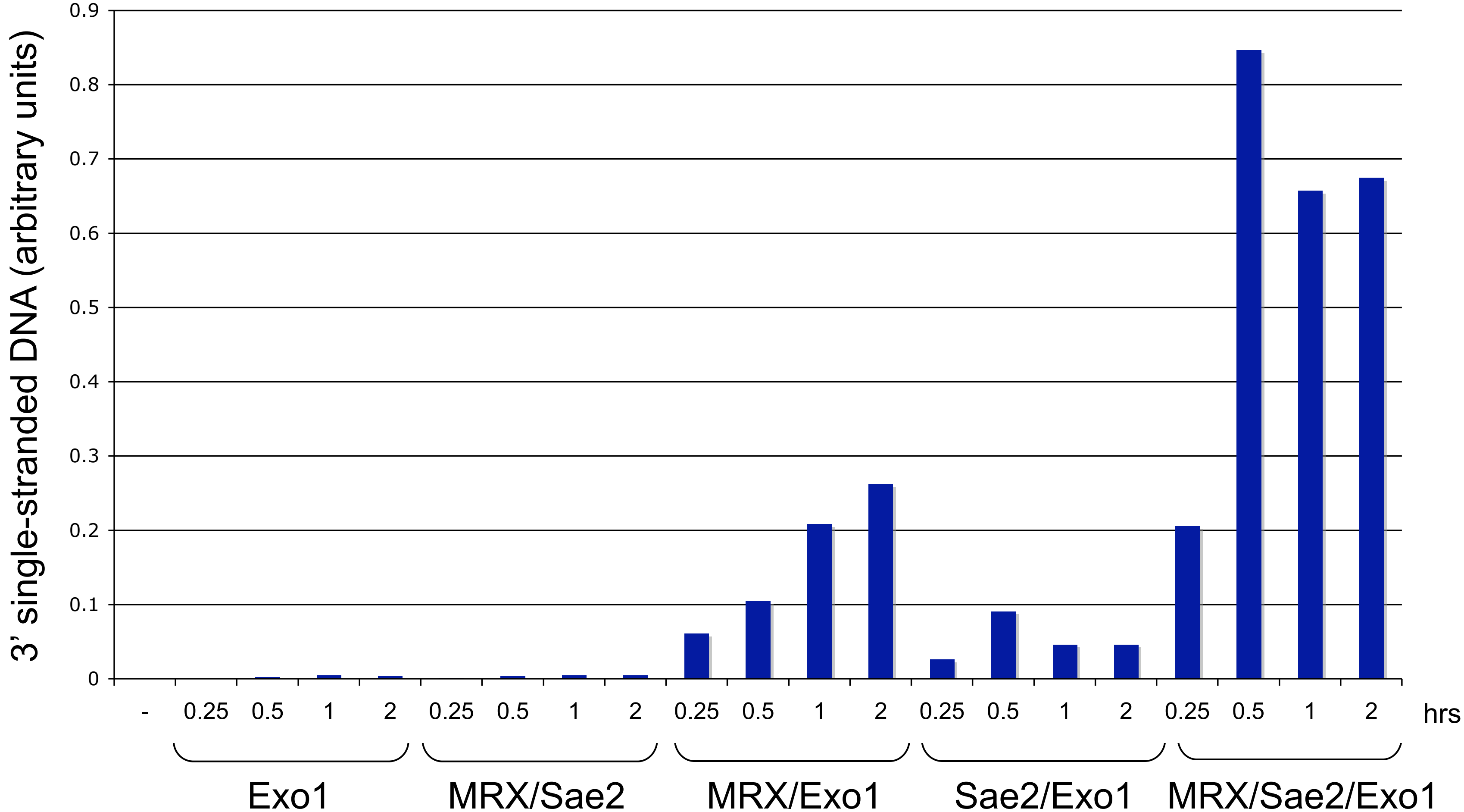

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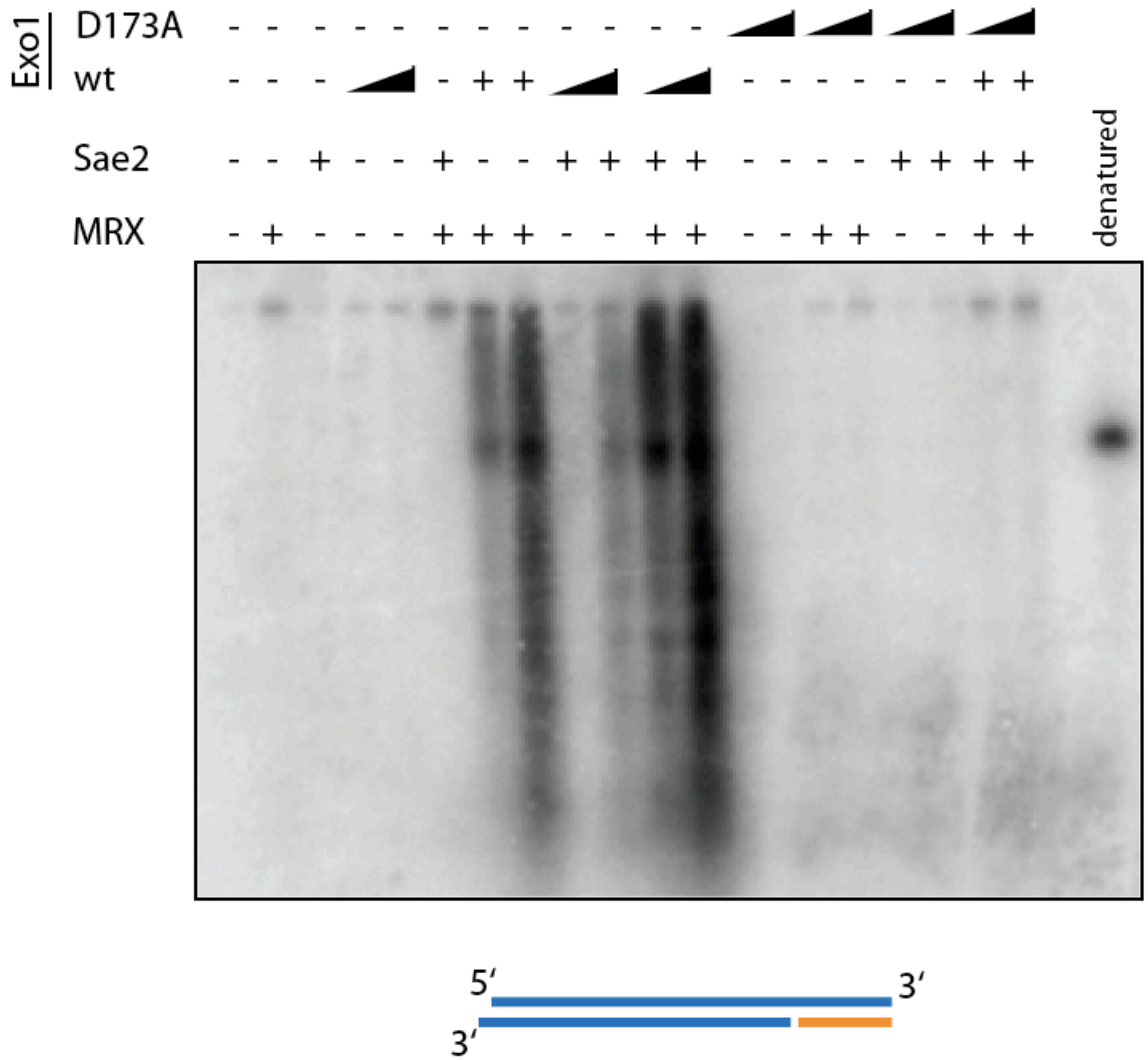
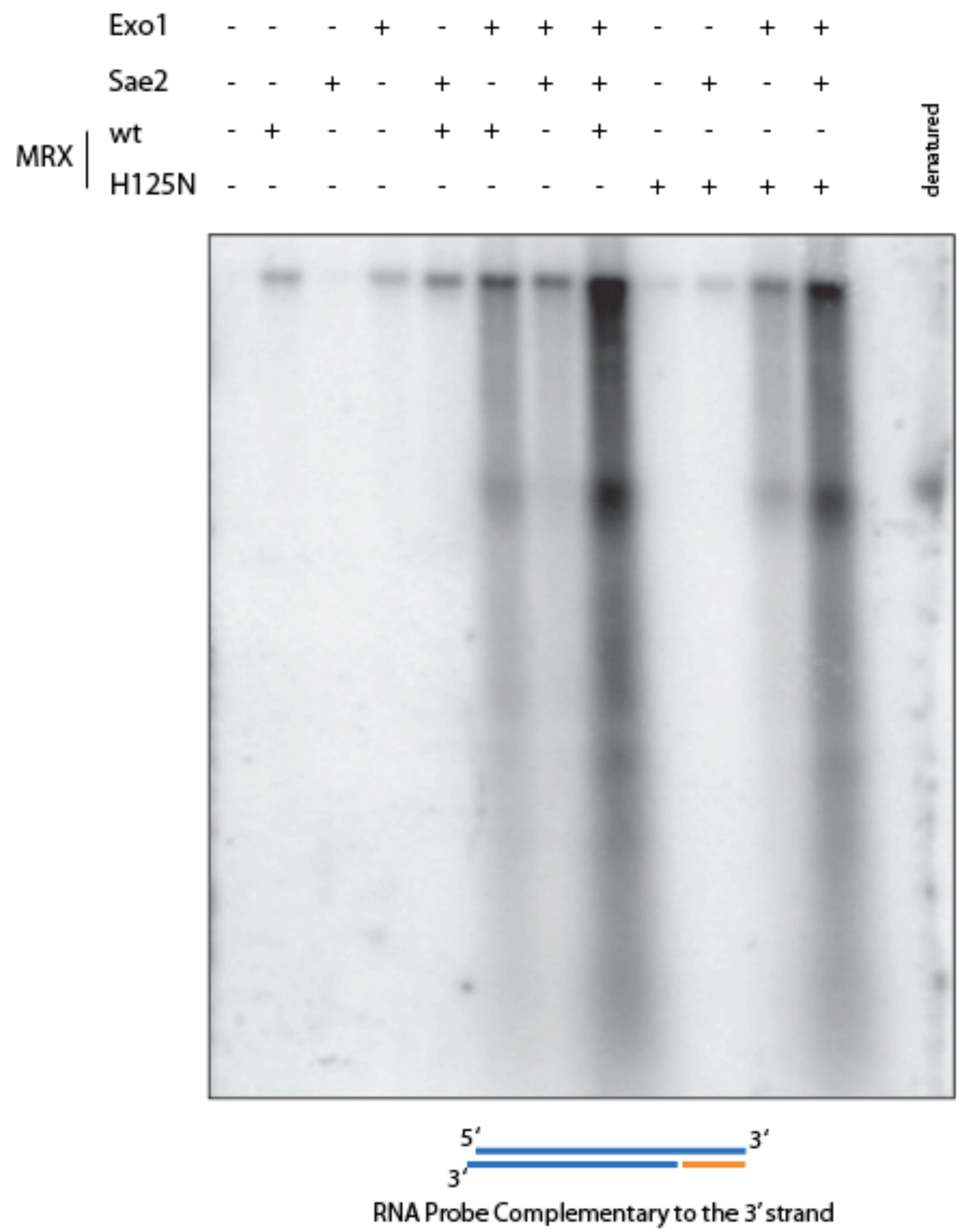
The catalytic activity of yExo1 is responsible for the resection:



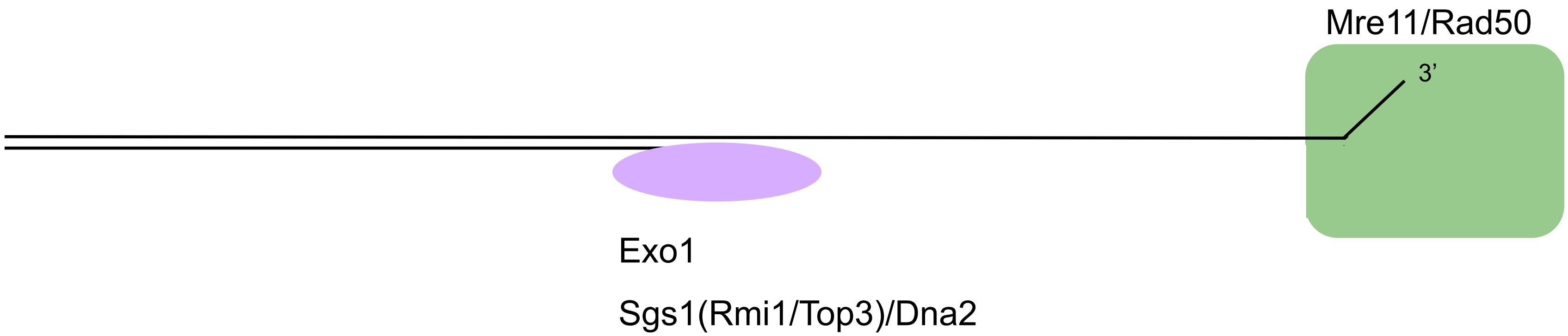
RNA Probe Complementary to the 3′ strand

Mre11 nuclease activity contributes only minimally to resection,

but reduces efficiency of initial cut that is dependent on MRX and Sae2



Working model for DSB processing by MRX/Sae2/Exo1:



? Is there a specific DNA structure that is formed by MRX that promotes the entry of or stimulates the catalytic activity of Exo1?

? How is resection controlled through CDK phosphorylation of Sae2?

? What is the function of Mec1/Tel1 phosphorylation of Sae2?

Functional orthologs of Sae2 in higher eukaryotes:

S. cerevisiae Sae2:	267	SPPGFGRLDFPSTQ	280
human CtIP:	847	TPENFWEVGFPSTQ	860
Xenopus CtIP:	806	TPENFWEVGFPSTQ	819
chicken CtIP:	862	TPENFWEVGFPSTQ	875
C. elegans Sae2/CtIP:	480	TPERYWDLTMGPRD	493

• CtIP/Ctp1 in human cells and in S. pombe is required for resistance to DNA-damaging agents as well for resection of DSBs and recruitment and activation of ATR

Sartori, A.A., Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., Baer, R., Lukas, J., and Jackson, S.P. (2007). Human CtIP promotes DNA end resection. Nature 450, 509-514.

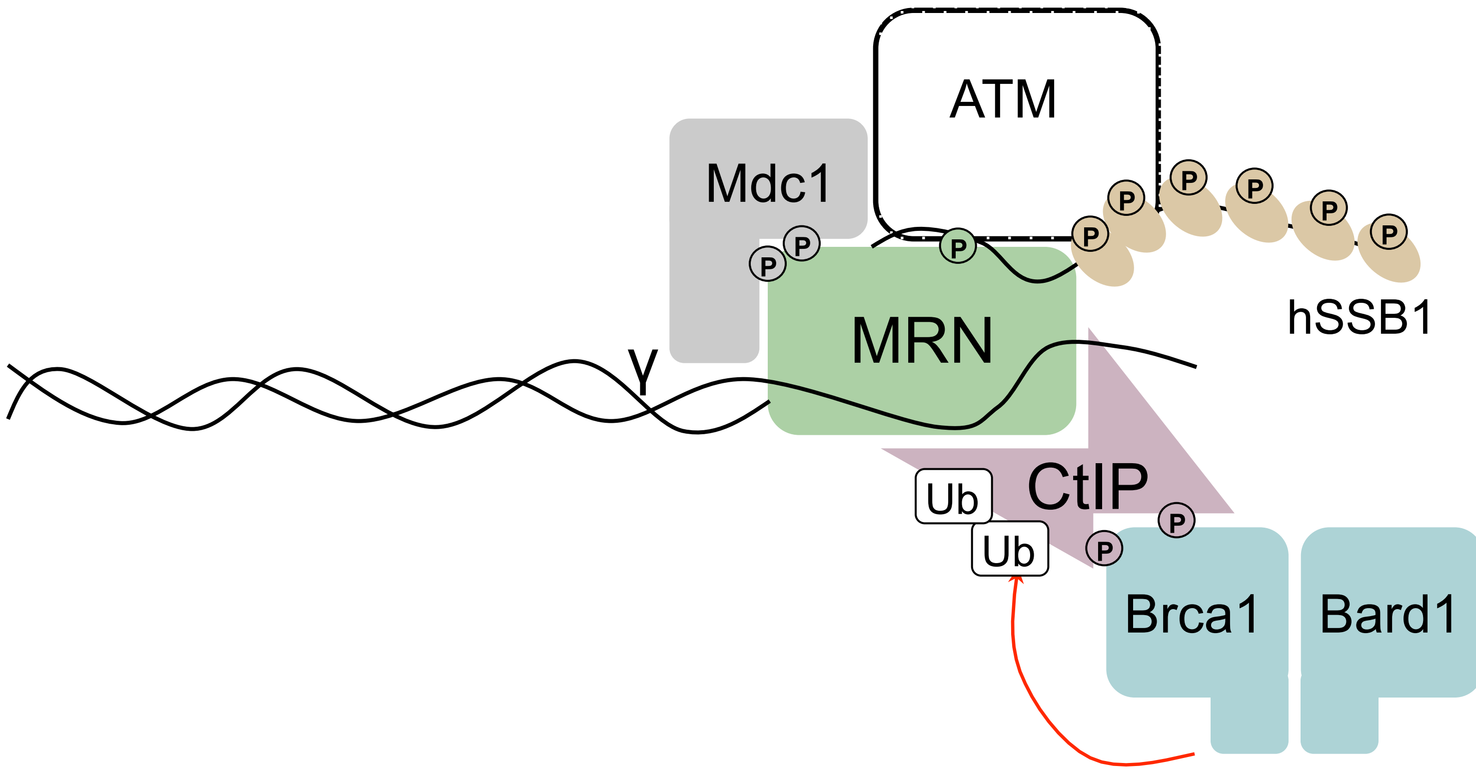
Limbo, O., Chahwan, C., Yamada, Y., de Bruin, R.A., Wittenberg, C., and Russell, P. (2007). Ctp1 is a cell-cycle-regulated protein that functions with Mre11 complex to control double-strand break repair by homologous recombination. Mol. Cell 28, 134-146.

• Com1/Sae2/CtIP in C. elegans and in Arabidopsis is required for meiotic DSB processing and chromosome pairing during meiosis

Penkner, A., Portik-Dobos, Z., Tang, L., Schnabel, R., Novatchkova, M., Jantsch, V., and Loidl, J. (2007). A conserved function for a Caenorhabditis elegans Com1/Sae2/CtIP protein homolog in meiotic recombination. EMBO J 26, 5071-5082.

Uanschou, C., Siwiec, T., Pedrosa-Harand, A., Kerzendorfer, C., Sanchez-Moran, E., Novatchkova, M., Akimcheva, S., Woglar, A., Klein, F., and Schlogelhofer, P. (2007). A novel plant gene essential for meiosis is related to the human CtIP and the yeast COM1/SAE2 gene. EMBO J 26, 5061-5070.

Multiple binding partners of MRN and CtIP at a DSB:



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